

**PATENTABILITY SEARCH  
RE "ENZYME ASSAY CHIP"**

**prepared by The Webb Law Firm**

**June, 2001**





WEBB ZIESENHEIM LOGSDON ORKIN & HANSON, P.C.

700 KOPPERS BUILDING

436 SEVENTH AVENUE

PITTSBURGH, PA 15219-1818

WILLIAM H. LOGSDON  
RUSSELL D. ORKIN  
DAVID C. HANSON  
FREDERICK B. ZIESENHEIM  
RICHARD L. BYRNE  
KENT E. BALDAUF  
BARBARA E. JOHNSON  
PAUL M. REZNICK  
JOHN W. McILVAINE III  
BLYNN L. SHIDELER

TELEPHONE 412-471-8815

FAX 412-471-4094

E-MAIL [webblaw@webblaw.com](mailto:webblaw@webblaw.com)

PATENT, TRADEMARK & COPYRIGHT LAW

JULIE W. MEDER  
LESTER N. FORTNEY  
RANDALL A. NOTZEN  
JAMES G. PORCELLI  
KENT E. BALDAUF, JR.  
CHRISTIAN E. SCHUSTER  
THOMAS J. CLINTON  
DEAN E. GEIBEL  
NATHAN J. PREPELKA  
JESSICA M. SOSENKO  
KIRK M. MILES  
J. MATTHEW PRITCHARD IV

WILLIAM H. WEBB (1929-1997)

June 8, 2001

PATENT AGENT  
GARY F. MATZ

F. J. Lucchino, Esq.  
General Counsel  
LaunchCyte LLC  
5001 Centre Avenue  
Pittsburgh, PA 15213

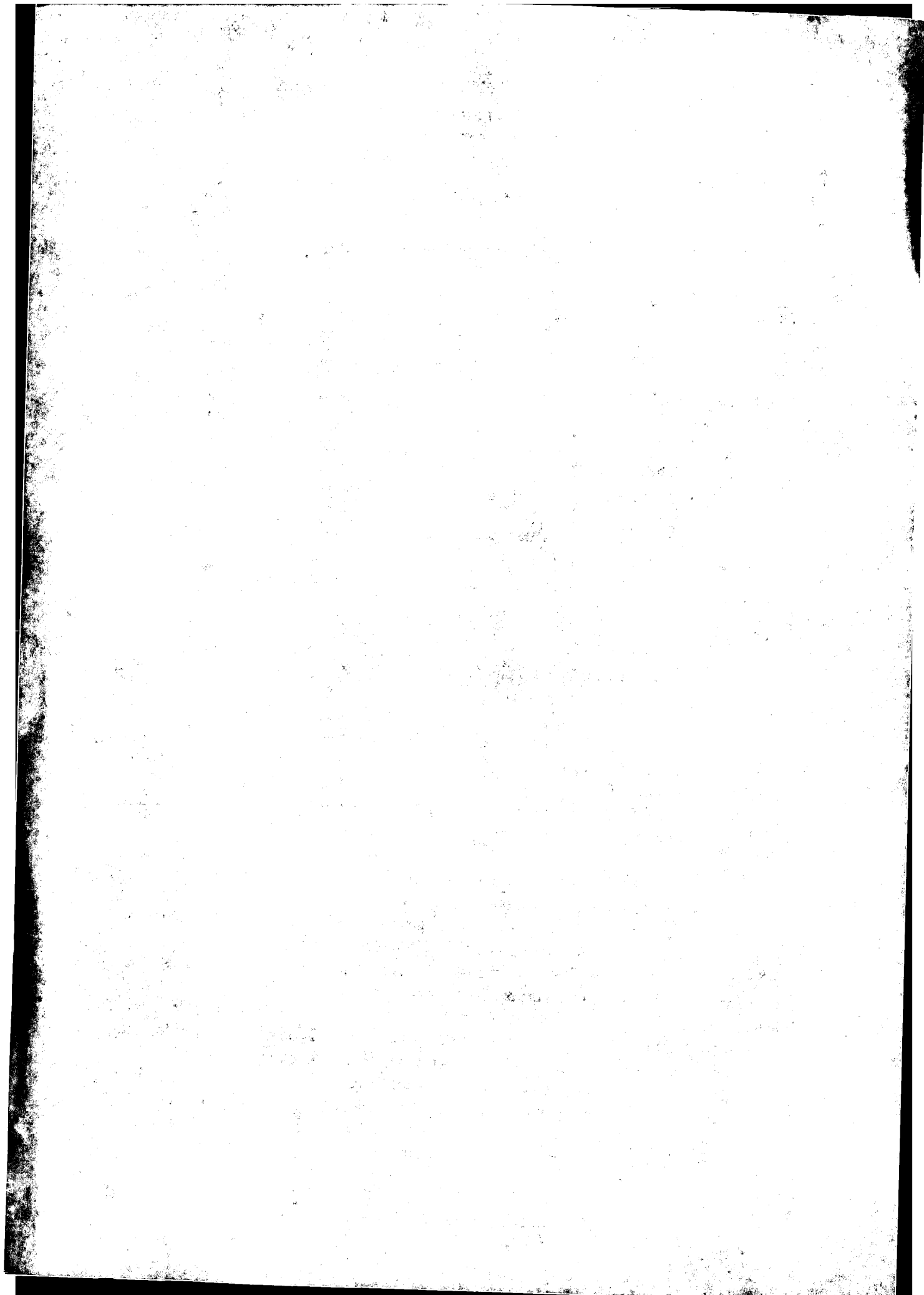
Re: Preliminary Patentability Search re Protein Chip Invention Disclosure by  
Dr. Scott Diamond  
Our File: 010140

Dear F.J.:

We have completed a preliminary patentability search directed to the protein chip disclosure provided to us. This letter reports the search strategy and search results to you.

Based upon the results of our search, it is our opinion that patent protection is available for (1) the deposition of hydrophilic microdots containing peptide or polymer onto a nonporous microarray substrate, taken in conjunction with (2) the method of applying the sample in misted or aerosolized form. Our opinion is based upon the absence, in the prior art identified by our search, of any disclosure or teaching of this combined subject matter.

Our search was directed to the following technology disclosed to us. Dots or microdots of one or more peptide or protein reagents are deposited onto a nonporous chip or slide, in which the peptide or protein reagent or reagents are suspended in a hydrophilic polymer carrier such as glycerol (or, in some cases, polyethylene glycol or other material). The dots or microdots are typically deposited by laser printing and are usually spaced apart in a scannable microarray. The sample to be contacted onto the microarray of dots is provided in a misted or aerosolized form, so that microaliquots of sample adhere to and penetrate into the microdots and migrate away from and/or evaporate from the areas between the microdots. The microdots may be used to conduct semi-quantitative chemical reactions visualized by way of chromagens or fluorogens in the microdot reactants. In completing the search, we bore in mind the possibility that the aerosolization of the sample may be created with ultrasonic energy, or similar energy capable of generating "nebulization" of the sample, preferably by means of apparatus which exerts ultrasonic energy to the sample without actually touching the sample. It is our



understanding that the predominant commercial embodiment for this technology will be used to test for a wide variety of enzymes and/or other constituents in a biological specimen. We further understand that the misting or aerosolization of the sample gives new and unexpected results in that it prevents cross-contamination between the microdots in addition to providing a controlled amount of sample for the purpose of realizing semi-quantitative and quantitative reactions in many cases.

Our search was conducted primarily in a number of commercial online databases available to us, and copies of the pertinent portions of the online printouts of those searches accompany this letter. The printout portions identify the databases which were searched and the keyword search statements used. Although a number of search strategies were implemented, all Dialog Information System databases were searched with the keyword search, "(microarray? or chip? or biochip?) and (protein? or peptid? or substrat?) and enzym? and (aerosol? or mist? or fog? or atomiz?) and scan?" in which "?" signifies variability truncation. In addition to the commercial databases, we also searched the U.S. Patents database available through the Internet home page for the United States Patent and Trademark Office (USPTO) web site. In the USPTO web site, we reviewed each of the 171 patents identified by the presence of the terms "peptide or protein" and "array or microarray or chip" in the claims of any U.S. patent. Recently issued U.S. patents in a number of manual classes and subclasses were reviewed in the search room of the United States Patent and Trademark Office in Washington, D.C.

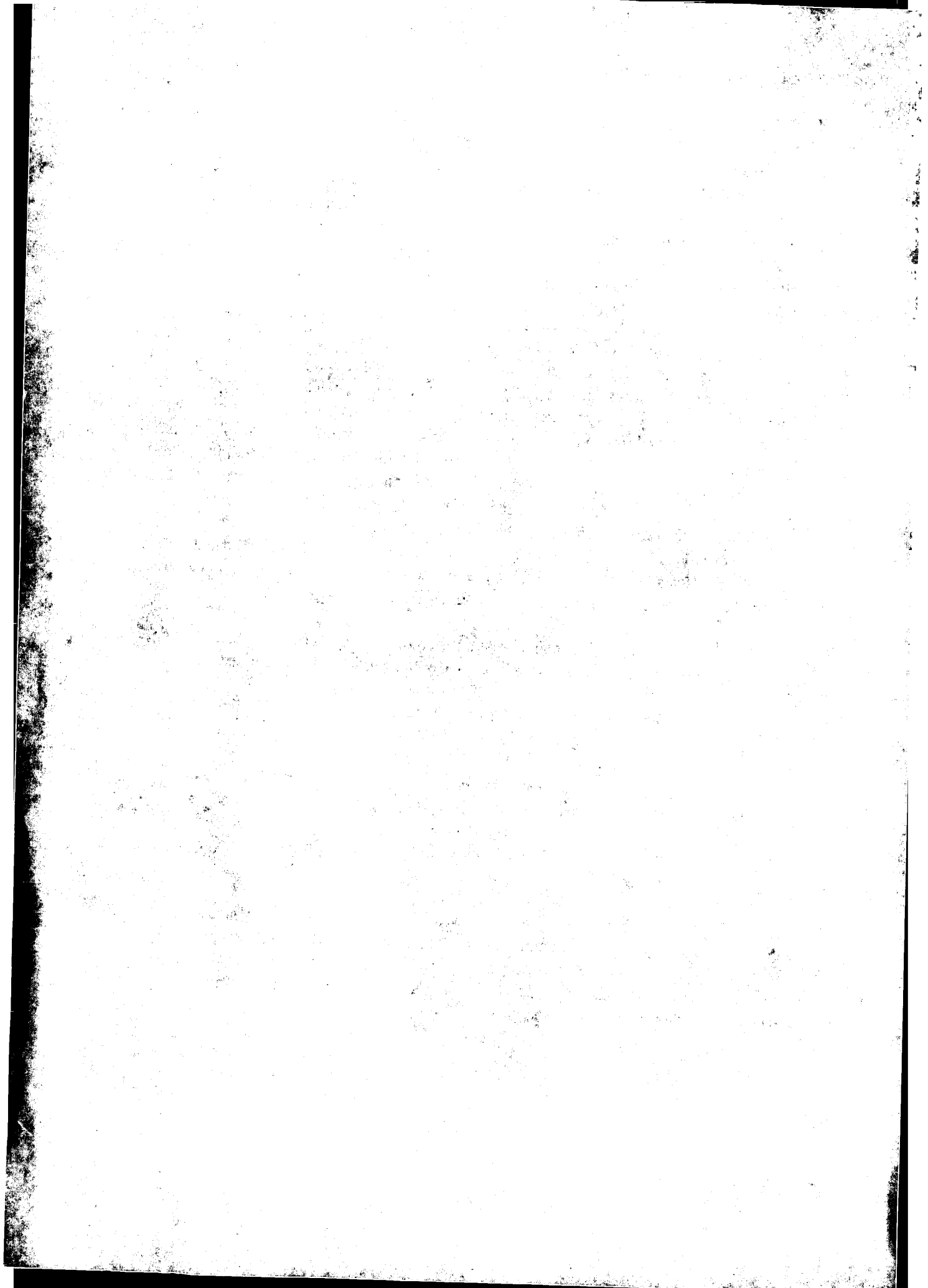
Several pertinent published articles (copies attached) were identified by our search, as summarized in the following list. Each of these articles is summarized briefly below.

Galvin MacBeath et al., "Printing Proteins as Microarrays for High-Throughput Function Determination," Science, vol. 289, pp. 1760-1763, September 8, 2000.

Gregory C. Adam et al., "Profiling the specific reactivity of the proteome with non-directed activity-based probes," Chemistry & Biology, vol. 57, pp. 1-16, 2000.

Zhu Heng et al., "Protein arrays and microarrays," Current Opinion in Chemical Biology, vol. 5, pp. 40-45, 2001.

A number of pertinent patents were identified by our search, which are identified in the list below and are attached herewith. Each of the accompanying pertinent patents is summarized briefly below. Additional prior art patents and publications which are not as pertinent as those listed above and below appear in the culled online database printouts accompanying this letter.



PATENT NO.	INVENTOR/S	DATE ISSUED
U.S. 4,046,513	Johnson	September 6, 1977
U.S. 5,985,551	Brennan	November 16, 1999
WO 00/04389	Wagner et al.	January 27, 2000
WO 00/04390	Wagner et al.	January 27, 2000
WO 00/54046	Ge	September 14, 2000
U.S. 6,225,061	Becker et al.	May 1, 2001

Zhu et al. disclose, in pertinent part, protein arrays confined to microwells or gel pads in order to segregate discrete reaction sites for protein reactions, also citing the earlier work of others. No misting or aerosolizing of the sample to be contacted onto the discrete reaction loci is taught.

MacBeath et al. disclose protein microarrays in which protein samples may be printed, in a glycerol carrier, onto slides, but the slides are first coated with a derivatizing agent, such as an aldehyde-containing silane. A quenching agent is overlaid on the slides to quench unreacted aldehydes, and other compensating reactions are also taught. MacBeath et al. do not suggest adhering droplets of glycerol and protein sample directly to a slide, nor is there any suggestion of misting or aerosolizing the sample to enhance sample application without cross-contamination.

Adam et al. represent a more generalized report of proteome reactivity than either Zhu et al. or MacBeath et al., and do not disclose glycerol dots of reactant(s) or misted or aerosolized sample application. Adam et al. is provided for technological background.

Published PCT Application WO 00/04389 to Wagner et al. (assigned on its face to Zyomyx, Inc.) discloses protein-capture arrays in a variety of configurations focusing on discrete patches for the protein-capture agents. The use of microdots or misting/aerosolizing of the biological sample is not taught or suggested.

Published PCT Application WO 00/04390 to Wagner et al. (assigned on its face to Zyomyx, Inc.) discloses microfabricated devices having biomolecules immobilized thereon, which biomolecules include polypeptides and proteins. The biomolecules are secured to the device by immobilization in a microchannel, which is generally fabricated into or onto a substrate.



Published PCT Application WO 00/54046 to Ge (assigned on its face to the National Institutes of Health) discloses what is purported to be a "universal protein array system." Protein arrays are disclosed, and emphasis is placed on the need for substrates (other than for example nitrocellulose and PVDF) having binding agents at the protein loci and blocking agents where protein binding is not desired. Deposition of glycerol/protein dots and misting/aerosolizing of the sample to be assayed are not taught or suggested by this published patent application.

U.S. Patent No. 6,225,061 to Becker et al. (assigned on its face to Sequenom, Inc.) discloses systems and methods for performing reactions in an unsealed environment. Dispensing pipettes deposit a submicroliter amount of a liquid to a target site, and evaporation is monitored and compensated for during the reaction. Notwithstanding the teaching regarding monitoring evaporation, there is no disclosure or suggestion of misting or aerosolizing a biological sample for application to a microarray of glycerol dots of protein or other reactants.

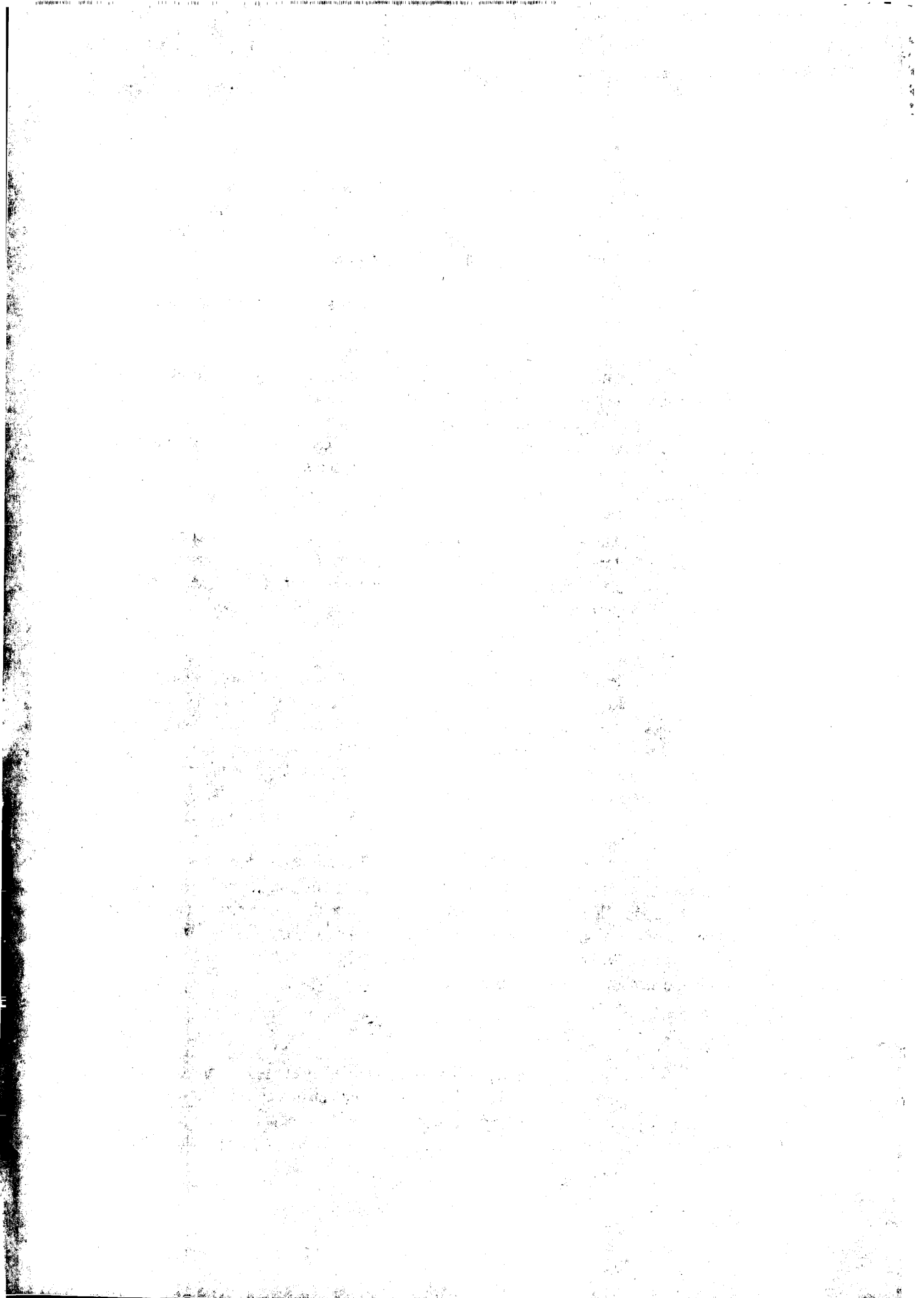
U.S. Patent No. 5,985,551 to Brennan (assigned on its face to Protogene Laboratories, Inc.) discloses apparatus and methods for making arrays of functionalized binding sites on a support surface. A particular binding method is used to bind chemical reactants by way of functionalized binding sites. Brennan does not disclose glycerol microdots or misted/aerosolized sample applications.

U.S. Patent No. 4,046,513 to Johnson (assigned on its face to Miles Laboratories, Inc.) entitled "Printed Reagent Test Devices and Method of Making Same" is included as an early patent of background interest. Johnson discloses a carrier matrix having discrete areas of reactant so that the reactants are maintained substantially separate from one another until the test device is wetted with the sample to be tested. Johnson does not disclose protein chips, glycerol dots, or the misting or aerosolization of a sample to be applied.

As we have discussed, it is not possible to search patent applications filed within approximately the last 20 months. As a result, it is possible that pertinent prior art which would affect the patentability of the above-described disclosure is not available for identification at this time. It is also possible, and should be borne in mind, that even the commercial databases used to complete this search cannot be counted on for complete integrity, and there is a small, but significant, possibility that certain prior art references or patents of interest could not have been noted within the databases or physical patent collections we searched.

### CONCLUSION

In view of the publications and patents identified by our search, and their inability to teach the combined creation of discrete microdot arrays and misting or aerosolization of the sample for application to the microdot arrays, we conclude that patent protection is available for the subject matter described above. We understand that a provisional patent application has





F. J. Lucchino, Esq.

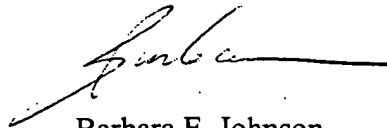
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June 8, 2001

already been filed by the University of Pennsylvania, and we look forward to working with you to convert the provisional patent application to a U.S. regular utility and/or Patent Cooperation Treaty (PCT) patent application, should you confirm that we should proceed.

We look forward to hearing from you.

Very truly yours,

A handwritten signature in dark ink, appearing to read 'Barbara E. Johnson', with a long horizontal flourish extending to the right.

Barbara E. Johnson

BEJ:mpr

Enclosures



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Welcome to DIALOG

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DIALOG(R)File 16:Gale Group PROMT(R)

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08276786 Supplier Number: 69846280 (THIS IS THE FULLTEXT)

**IBC Presents the First Conference Dedicated to Protein Microarray  
Technology.**

PR Newswire, p4379

Feb 2, 2001

TEXT:

WESTBOROUGH, Mass., Feb. 2 /PRNewswire/ --

IBC USA organizes Protein Microarray Technology: From Proteomics  
Discovery to Diagnostics -- Expectations and Limitations. The event will  
be held on March 21-23, 2001 in San Diego, CA.

Microarray technology allows the simultaneous analysis of thousands

of molecular parameters with a single experiment. Today, methods and techniques of the DNA chip field are being effectively transferred to protein array technology. This event allows attendees to learn about where this new, exciting field is at right now, where it is moving, and how proteomics, drug discovery, and diagnostics are being revolutionized by protein microtechnology. This event aims to accelerate discussion of The Proteome Project and examine the technologies being developed and how they will impact research.

Conference highlights include:

\*Keynote address by Dr. Roger Ekins - a founding father of microtechnology

\*Data from Beta Tests, as well as Commercial Applications

\*How to make and use protein/peptide arrays

\*Direct application of technology in diagnostics

\*Proteomics discovery research and assay development

\*Latest technologies in the protein array field

\*First-time presentations of case study data/results

\*Bridging the gap between genomics and proteomics

For more details on this, visit our website:

<http://www.ibcusa.com/2623>.

CONTACT:

Abby Votto

IBC USA Conferences

One Research Drive

P.O. Box 5195

Suite 400A

Westborough, MA 01581-5195

[avotto@ibcusa.com](mailto:avotto@ibcusa.com)

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DIALOG(R)File 16:Gale Group PROMT(R)  
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07919803 Supplier Number: 66188363 (THIS IS THE FULLTEXT)  
**Oxford Glycosciences and Cambridge Antibody Technology announce strategic alliance in protein microarrays. (Brief Article)**  
BIOTECH Patent News, n9, pNA  
Sept, 2000

TEXT:

Oxford GlycoSciences plc (Oxfordshire, England; +44-1235-543200) and Cambridge Antibody Technology (Melbourn, England; +44-1763-263233) announced the formation of a collaboration to develop "protein chip" technology for detection of proteins using antibody based microarrays.

A protein chip is a chemically modified surface onto which a high density of protein-based probes have been precisely applied in an ordered manner. A microarray is a chemically modified surface (chip) onto which a high density of probes have been precisely applied in an ordered manner.

The companies will combine their respective technologies, to develop a new protein detection and screening technology based on antibody microarrays. Oxford's human protein libraries and Cambridge Antibody's human antibody libraries will be analysed, paired proteins and antibodies selected and microarrays developed based on Oxford's current protein microarray prototype format. The goal is to create a new generation of protein detection technology with the speed, throughput and sensitivity to serve the development of research tools, diagnostics and novel therapeutics. Each party will fund its own research contribution.

Michael Kranda, CEO of Oxford said, "With the Human Genome map in hand the spotlight has shifted to proteins, proteomics and antibody technology. The Cambridge Antibody/Oxford Protein Chip project will focus on combining our expertise to explore the next generation of high throughput proteomics technology. We see the field of analytical proteomics maturing similarly to genomics. Oxford pioneered industrialised proteomics based on 2D gels and mass spectrometry, for discovery of human proteins. We currently have over 800 patent filings covering disease specific proteins, with the number growing. Our technology team has used our expertise in proteomics, micro-engineering and informatics, to develop the prototype of an antibody-based protein detection microarray. The alliance with Cambridge Antibody provides us access to their high throughput antibody technology to bring our prototype micro arrays to an industrial scale for developing protein chips as screening and diagnostic tools. We are very pleased to link up with the acknowledged antibody technology expertise at Cambridge Antibody."

David Chiswell, CEO of Cambridge Antibody said, "Understanding the expression, processing, modification and activity of proteins is fundamental to the development of revolutionary diagnostics, prognostics

and therapies for human disease management. We at Cambridge Antibody believe that antibody-based microarrays will be a core tool for unlocking this understanding. Cambridge Antibody's extensive pioneering work on high throughput antibody isolation, which has included the world's first fully automated, high throughput antibody selection and screening processes developed three years ago, makes Cambridge Antibody the partner of choice to develop Protein Chips. Teaming up with the world-leading proteomics expertise at Oxford GlycoSciences is Cambridge Antibody's first collaboration designed to help solve the technological and commercial challenges in developing Protein Chips and to unlock the strategic value in this new market."

Oxford is the world's leading proteomics based drug discovery and development company, applying its proteomics technology to pharmaceutical research and development. Oxford has proteomics collaborations with a number of major pharmaceutical companies, including programmes with Pfizer in Alzheimer's disease and atherosclerosis, with Merck in diabetes, and in respiratory disease with Bayer. Oxford also has a proteomics programme with Pioneer Hi-Bred/DuPont. Furthermore, in a joint venture with the genomics company, Incyte Genomics Inc., Oxford is building expression databases for licence to pharmaceutical companies, to which Astra Zeneca became the first subscriber in 1999.

Oxford has drug discovery programmes in oncology, inflammation, and infectious disease, and a growing clinical and pre-clinical pipeline. OGT 918 (Vesvesca) is in late stage clinical trials for the treatment of glycolipid storage disease.

Cambridge Antibody is a biotechnology company using its proprietary technologies in fully human monoclonal antibodies for drug discovery and drug development. Cambridge Antibody currently employs around 180 people. Cambridge Antibody has a world leading platform technology for rapidly isolating fully human monoclonal antibodies using proprietary phage display systems. Cambridge Antibody has an extensive phage display antibody library, currently incorporating around 100 billion distinct antibodies. This library forms the basis for the company's strategy to develop a portfolio of clinical development programmes and for discovering new drug leads using functional genomics. Four fully human therapeutic antibodies developed by CAT are at various stages of clinical trials.

Cambridge Antibody has a number of license and collaborative agreements in place with pharmaceutical and biotechnology companies including: Eli Lilly, Pfizer, BASF Pharma, Genentech, ICOS Corporation, Genetics Institute, Wyeth-Ayerst, Human Genome Sciences, AstraZeneca and Pharmacia.

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Logon file001 25jan01 12:28:41

\*\*\* ANNOUNCEMENT \*\*\*

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NEW FILE RELEASED

\*\*\*Investext PDF Index (File 745)

\*\*\*Daily and Sunday Telegraph (London) Papers (File 756)

\*\*\*The Mirror Group Publications (United Kingdom) (File 757)

UPDATING RESUMED

\*\*\*Extel News Cards from Primark (File 501)

\*\*\*TFSD Ownership Database (File 540)

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RELOADED

\*\*\*Kompas Central/Eastern Europe (File 593)

\*\*\*Kompas Latin America (File 586)

\*\*\*Brands and their Companies (File 116)

\*\*\*Kompas USA (File 584)

\*\*\*Kompas Canada (File 594)

\*\*\*PsyncINFO (File 11)

FILES REMOVED

\*\*\*EconBase (File 565)

\*\*\*Unlisted Drugs (File 140)

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\$0.19 Estimated cost File1

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\$0.33 Estimated cost this search

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File 652:US Patents Fulltext 1971-1979

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\*File 652: Reassignment data current through 7/25/2000 recordings.  
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File 653:US Patents Fulltext 1980-1989

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\*File 653: Reassignment data current through 7/25/2000 recordings.  
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File 654:US Pat.Full. 1990-2001/Jan 23

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\*File 654: Reassignment data current through 12/5/2000 recordings.

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7 MICROARRAY/TI

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1/5/1 (Item 1 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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03244046

METHODS FOR DETERMINING CROSS-HYBRIDIZATION

PATENT NO.: 6,171,794

ISSUED: January 09, 2001 (20010109)

INVENTOR(s): Burchard, Julja, Kirkland, WA (Washington), US (United States  
of America)

Stoughton, Roland, San Diego, CA (California), US (United  
States of America)

Friend, Stephen H., Seattle, WA (Washington), US (United  
States of America)

ASSIGNEE(s): Rosetta Inpharmatics, Inc., (A U.S. Company or Corporation),  
Kirkland, WA (Washington), US (United States of America)

[Assignee Code(s): 51113]  
APPL. NO.: 9-335,971  
FILED: June 18, 1999 (19990618)

This application claims benefit under 35 U.S.C. selection 119(e) of copending U.S. provisional patent application Ser. No. 60-092,512 filed on Jul. 13, 1998 which is incorporated herein by reference in its entirety.

U.S. CLASS: 435-6 cross ref: 435-287.2; 536-23.1; 536-24.31

INTL CLASS: [7] C12Q 1-68; C12M 1-34; C07H 21-07

FIELD OF SEARCH: 435-6; 435-6.6; 435-287.1; 536-23.1; 536-24.31

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5,556,752	9/1996	Lockhart et al.
5,569,588	10/1996	Ashby et al.
5,578,832	11/1996	Trulson et al.
5,599,668	2/1997	Stimpson et al.

##### NON-U.S. PATENT DOCUMENTS

WO 98-12354	3/1998	WO (World Intellectual Property Org)
WO 98-41531	9/1998	WO (World Intellectual Property Org)

##### OTHER REFERENCES

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PRIMARY EXAMINER: Zitomer, Stephanie W.  
ATTORNEY, AGENT, OR FIRM: Pennie & Edmonds LLP  
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#### ABSTRACT

The present invention provides methods for distinguishing the fractions of polynucleotide sequences which hybridize to any given probe, including probes on microarrays such as those described herein. In particular, the present invention enables users to identify the fraction of sequences which are perfectly complementary to a probe, thereby correcting for effects of cross hybridization in a hybridization assay. The methods of the invention work by monitoring the kinetics of dissociation of sequences from the probe so that a resulting "dissociation curve" may be compared to a combination of the individual "dissociation profiles" for each sequence which

hybridizes. In alternative embodiments, the invention also provides computer systems for performing the present methods, as well as databases of the dissociation profiles.

What is claimed is:

1. A method for determining a contribution of a reference polynucleotide to hybridization of polynucleotides in a sample to a test probe that is immobilized on a first solid surface, said method comprising comparing a dissociation curve for said hybridization to a dissociation profile for the reference polynucleotide, wherein:
  - (a) the dissociation curve is provided by a method comprising
    - (i) contacting a sample to the test probe under conditions that allow polynucleotides in the sample to hybridize to the test probe,
    - (ii) repeatedly washing the test probe under conditions such that some fraction of the hybridized polynucleotides dissociates from the test probe, and
    - (iii) measuring hybridization of the polynucleotides in the sample to the test probe after each of a plurality of said washing steps, over a time period wherein a detectable fraction of hybridized polynucleotides dissociates from the test probe; and
  - (b) the dissociation profile is provided by a method comprising
    - (i) contacting the reference polynucleotide to a reference probe that is immobilized on a second solid surface under conditions that allow the reference polynucleotide to hybridize to the reference probe,
    - (ii) repeatedly washing the reference probe under conditions such that some fraction of the hybridized polynucleotides dissociates from the reference probe, and
    - (iii) measuring hybridization of the reference polynucleotide to the reference probe after each of a plurality of said washing steps, over a time period wherein a detectable fraction of the polynucleotide dissociates from the reference probe; and
- wherein the contribution of the reference polynucleotide to hybridization to the test probe is the contribution of the dissociation profile to the dissociation curve.
2. The method of claim 1, wherein the reference probe is identical to the test probe.
3. The method of claim 1, wherein the test probe and the reference probe are the same probe.
4. The method of claim 3, wherein the step of contacting a sample to the test probe is performed concurrently with the step of contacting the reference polynucleotide to the reference probe.
5. The method of claim 3 wherein the step of contacting a sample to the test probe is performed at a different time than the step of contacting the reference polynucleotide to the reference probe.
6. The method of claim 1 wherein said comparing comprises minimizing the value of an objective function of the difference between the dissociation curve and the dissociation profile.
7. The method of claim 6 wherein the objective function is an absolute square of the difference of the dissociation curve and the dissociation profile.
8. The method of claim 6 wherein the objective function is a chi-squared quantity.
9. The method of claim 1, wherein the reference polynucleotide is detectably labeled.
10. The method of claim 9, wherein the detectable label is a fluorescent label.
11. The method of claim 10, wherein the fluorescent label is fluorescein, rhodamine, texas red, or a derivative thereof.

12. The method of claim 10, wherein the fluorescent label is FAM, JOE, ROX, HEX, TET, IRD40, IRD41, a cyanine dye, a BODIPY dye, or an ALEXA dye.

13. The method of claim 12, wherein the cyanine dye is selected from the group consisting of Cy3, Cy3.5, and Cy5.

14. The method of claim 12, wherein the BODIPY dye is selected from the group consisting of BODIPY-FL, BODIPY-TR, BODIPY-TMR, BODIPY-630/650, and BODIPY-650/670.

15. The method of claim 12, wherein the ALEXA dye is selected from the group consisting of ALEXA-488, ALEXA-532, ALEXA-546, ALEXA-568, and ALEXA-594.

16. The method of claim 9, wherein the detectable label is a radioactive isotope.

17. The method of claim 16, wherein the radioactive isotope is selected from the group consisting of sup 32 P, sup 35 S, sup 14 C, and sup 125 I.

18. The method of claim 9, wherein the detectable label is an electron rich molecule.

19. The method of claim 18, wherein the electron rich molecule is selected from the group consisting of ferritin, hemocyanin, and colloidal gold.

20. The method of claim 9, wherein the detectable label comprises a first chemical group specifically complexed to the reference polynucleotide, wherein said first chemical group is detected by contacting the first chemical group with a second chemical group; and wherein said second chemical group:

(i) has binding affinity for the first chemical group, and

(ii) is covalently linked to an indicator molecule.

21. The method of claim 9, wherein the detectable label is biotin or imminobiotin.

22. The method of claim 1, wherein the reference polynucleotide is a naturally occurring nucleic acid.

23. The method of claim 22, wherein the naturally occurring nucleic acid molecules are genomic DNA isolated from cells or an organism.

24. The method of claim 22, wherein the naturally occurring nucleic acid is RNA isolated from cells or an organism.

25. The method of claim 1, wherein the reference polynucleotide is RNA expressed by a cell or organism, cDNA derived therefrom, or crNA derived from said cDNA.

26. The method of claim 25, wherein the RNA is messenger RNA.

27. The method of claim 1, wherein the reference polynucleotide is a synthetic nucleic acid.

28. The method of claim 1, wherein the polynucleotide is cDNA.

29. The method of claim 1, wherein the reference polynucleotide is a polynucleotide synthesized by polymerase chain reaction.

30. The method of claim 1, wherein the test probe comprises a DNA sequence.

31. The method of claim 30, wherein the DNA sequence of the test probe comprises a genomic DNA sequence.

32. The method of claim 30, wherein the DNA sequence of the test probe comprise a cDNA sequence.

33. The method of claim 1, wherein the test probe comprises an RNA sequence.
34. The method of claim 33, wherein the RNA sequence of the test probe comprises a messenger RNA sequence.
35. The method of claim 1, wherein the test probe comprises a sequence of DNA analogues.
36. The method of claim 1, wherein the test probe comprises a sequence of RNA analogues.
37. The method of claim 1, wherein the first solid surface and the second solid surface are each a cellulose filter.
38. The method of claim 1, wherein the first solid surface and the second solid surface are each a nylon membrane.
39. The method of claim 1, wherein the first solid surface and the second solid surface are each a glass surface.
40. The method of claim 1, wherein the first solid surface and the second solid surface are each a nonporous surface.
41. The method of claim 1, wherein the first solid surface and the second solid surface are each a porous surface.
42. The method of claim 1, wherein the test probe is part of an array of probes.
43. The method of claim 42, wherein the array of probes is a microarray.
44. The method of claim 43, wherein the probes of the microarray are complementary to fewer than 50% of the genes in the genome of an organism.
45. The method of claim 43, wherein the probes of the microarray are complementary to at least 50% of the genes in the genome of an organism.
46. The method of claim 45, wherein the probes of the microarray are complementary to at least 75% of the genes in the genome of an organism.
47. The method of claim 45, wherein the probes of the microarray are complementary to at least 85% of the genes in the genome of an organism.
48. The method of claim 45, wherein the probes of the microarray are complementary to at least 90% of the genes in the genome of an organism.
49. The method of claim 45, wherein the probes of the microarray are complementary to at least 99% of the genes in the genome of an organism.
50. The method of claim 43, wherein each probe of the microarray comprises a polynucleotide sequence of between 20 bases and 50,000 bases.
51. The method of claim 50, wherein each probe of the microarray comprises a polynucleotide sequence of between 100 bases and 2,000 bases.
52. The method of claim 51, wherein each probe of the microarray comprises a polynucleotide sequence of between 300 bases and 1,000 bases.
53. The method of claim 43, wherein the density of the probes of the microarray is greater than 60 different probes per cm<sup>2</sup>.
54. The method of claim 43, wherein the probes of the microarray comprise single stranded oligonucleotides of 10 to 50 bases.
55. The method of claim 43, wherein the probes of the microarray comprise single stranded oligonucleotides of greater than 50 bases.
56. The method of claim 43, wherein the probes of the microarray comprise single stranded polynucleotides of greater than 100 bases.



57. The method of claim 43, wherein the probes of the microarray comprise Sequence-Tagged Sites.

58. The method of claim 1, wherein the step of contacting the reference polynucleotide to the reference probe is carried out by a method comprising contacting a reference sample to the reference probe,  
said reference sample comprising molecules of a plurality of different polynucleotides,  
said plurality of different polynucleotides comprising the reference polynucleotide, and  
each of said different polynucleotides being differentially labeled.

59. The method of claim 3, wherein:  
polynucleotides in the sample contacted to the test probe are labeled with a first label, and  
the reference polynucleotide contacted to the reference probe is labeled with a second label, said second label being distinguishable from the first label.

60. A method for determining a contribution of a reference polynucleotide to hybridization of polynucleotides in a sample to a test probe that is immobilized on a solid surface, said method comprising comparing a dissociation curve for said hybridization to a dissociation profile for the reference polynucleotide, in which:

the dissociation curve is provided by a method comprising  
(a) contacting a sample to the test probe under conditions that allow polynucleotides in the sample to hybridize to the test probe;  
(b) repeatedly washing the test probe under conditions such that some fraction of the hybridized polynucleotides dissociates from the test probe; and  
(c) measuring hybridization of the polynucleotides in the sample to the test probe after each of a plurality of said washing steps, over a time period wherein a detectable fraction of hybridized polynucleotides dissociates from the test probe;  
wherein the dissociation profile is provided from a theoretical prediction of a dissociation time,  $t_{\text{diss}}$ , of the reference polynucleotide from a reference probe provided by: [See equation in original document]9##

wherein  $R$  is the ideal gas constant,  $T$  is the temperature in degrees Kelvin,  $\Delta G$  is the binding energy, and  $\alpha$  and  $\beta$  are fitting parameters that are fit to experimental data; and

wherein the contribution of the polynucleotide to molecules hybridized to the test probe is the contribution of the dissociation profile to the dissociation curve.

61. The method of claim 60, wherein the dissociation profile is an exponential decay function.

62. The method of claim 1 or 60, wherein the reference polynucleotide hybridizes to the test probe with no base mismatches.

63. A method for determining the contribution of each of a plurality of polynucleotides to hybridization of polynucleotides in a sample to a test probe that is immobilized on a first solid surface, said method comprising comparing a dissociation curve for said hybridization to a dissociation profile for each of the plurality of polynucleotides, in which:

(a) the dissociation curve is provided by a method comprising  
(i) contacting a sample to the test probe under conditions that allow polynucleotides in the sample to hybridize to the test probe;  
(ii) repeatedly washing the test probe under conditions such that some fraction of the hybridized polynucleotides dissociates from the test probe; and  
(iii) measuring hybridization of polynucleotides in the sample to the test probe after each of a plurality of said washing steps, over a time period wherein a detectable fraction of hybridized polynucleotides dissociates from the test probe, and

(b) the dissociation profile for each of the plurality of polynucleotides is provided by a method comprising

(i) contacting each of the plurality of polynucleotides to a reference probe that is immobilized on a second solid surface under conditions that

allow each of the plurality of polynucleotides to hybridize to the reference probe;

(ii) repeatedly washing the reference probe under conditions such that some fraction of the hybridized polynucleotides dissociates from the reference probe; and

(iii) measuring hybridization of each of the plurality of polynucleotides to the reference probe after each of a plurality of said washing steps, over a time period wherein a detectable fraction of each of the plurality of polynucleotides dissociates from the reference probe,

wherein the contribution of each of the different polynucleotide molecules to hybridization to the test probe is the contribution of the dissociation profile for each of the plurality of polynucleotides to the dissociation curve.

64. The method of claim 63, wherein each of the plurality of polynucleotides has a different polynucleotide sequence.

65. The method of claim 63, wherein each of the plurality of polynucleotides is contacted separately to the reference probe.

66. The method of claim 63, wherein each of the plurality of polynucleotides is differentially labeled.

67. The method of claim 66, wherein each of the plurality of polynucleotides is contacted concurrently to the reference probe.

68. A method for determining the contribution of each of a plurality of polynucleotides to hybridization of polynucleotides in a sample to a test probe that is immobilized on a solid surface, said method comprising comparing a dissociation curve for said hybridization to a dissociation profile for each of the plurality of polynucleotides, in which:

(a) the dissociation curve is provided by a method comprising

(i) contacting a sample to the test probe under conditions that allow polynucleotides in the sample to hybridize to the test probe;

(ii) repeatedly washing the test probe under conditions such that some fraction of the hybridized polynucleotides dissociates from the test probe; and

(iii) measuring hybridization of the polynucleotides in the sample to the test probe after each of a plurality of said washing steps, over a time period wherein a detectable fraction of hybridized polynucleotides dissociates from the test probe, and

(b) the dissociation profile for each of the plurality of polynucleotides is provided from a theoretical prediction of a dissociation time,  $t_{\text{diss}}$ , for each of the plurality of polynucleotides from a reference probe provided by: [See equation in original document]10##

wherein  $R$  is the ideal gas constant,  $T$  is the temperature in degrees Kelvin,  $\Delta G$  is the binding energy and  $\alpha$  and  $\beta$  are fitting parameters that as fit to experimental data,

wherein the contribution of each of the plurality of polynucleotides to molecules hybridized to the test probe is the contribution of the dissociation profile for each of the plurality of polynucleotides to the dissociation curve.

69. The method of claim 68, wherein the form of the dissociation profile for each of the plurality of polynucleotides is an exponential decay function.

70. The method of claim 63 or 68 wherein said comparing comprises minimizing the value of an objective function of the difference between the dissociation curve and the dissociation profiles.

71. The method of claim 70 wherein the objective function is an absolute square of the difference of the dissociation curve and a combination of the dissociation profiles.

72. The method of claim 71 wherein the combination of the dissociation

profiles is a summation of the one or more dissociation profiles.

73. The method of claim 70 wherein the objective function is a chi-squared quantity.

74. The method of claim 25 wherein the reference polynucleotide is cRNA.

75. The method of claim 1, wherein said first solid surface and said second solid surface are the same solid surface.

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Utility  
MONITORING TOXICOLOGICAL RESPONSES

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INVENTOR(s): Cunningham, Mary Jane, Sunnyvale, CA (California), US (United States of America)  
Zweiger, Gary B., San Mateo, CA (California), US (United States of America)  
Panzer, Scott R., Sunnyvale, CA (California), US (United States of America)  
Seilhamer, Jeffrey J., Los Altos Hills, CA (California), US (United States of America)  
ASSIGNEE(s): Incyte Pharmaceuticals, Inc., (A U.S. Company or Corporation), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 27511]  
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Hawashima H. et al., Protein Expression, Characterization, and

PRIMARY EXAMINER: Jones, W. Gary  
ASST. EXAMINER: Siew, Jeffrey  
ATTORNEY, AGENT, OR FIRM: Incyte Genomics, Inc.  
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ART UNIT: 166

#### ABSTRACT

The present invention relates to a composition comprising a plurality of polynucleotide targets. The composition can be used as hybridizable array elements in a **microarray**. The present invention also relates to methods for screening compounds and therapeutic treatments for toxicological responses.

What is claimed is:

1. A method for detecting a toxicological effect of a test compound associated with increased or decreased levels of a polynucleotide sequence in a sample comprising:
  - a) treating a sample with a toxin;
  - b) obtaining a polynucleotide sequence from the sample treated with the toxin;
  - c) contacting the polynucleotide sequence with an array comprising a combustion of polynucleotides comprising SEQ ID NOs: 1-61 under conditions effective to form one or more hybridization complexes;
  - d) detecting the hybridization complexes, wherein the presence, absence or change in amount of the hybridization complex, as compared with the hybridization complexes formed by SEQ ID NOs. 1-61 with a polynucleotide sequence from an untreated sample, is indicative of a toxicological response to the toxin; then
  - e) measuring the level of polynucleotide sequences in a sample treated with a test compound using the method of steps (c) and (d); and
  - f) comparing the level detected in step (e) with the level detected in step (d) wherein an increase or decrease in the level detected in step (e) indicates a toxicological response to the test compound.
2. The method of claim 1, wherein the sample is a tissue selected group consisting of liver, kidney, brain, spleen pancreas, and lung.
3. The method of claim 1, wherein the toxin is acetaminophen or its metabolites.
4. The method of claim 1, wherein the test compound which elicits the toxicological response induces at least a 2.5 fold change in the amount of the hybridization complexes formed between the polynucleotide of the array with at least one of the polynucleotide sequences of the sample, when compared with polynucleotide sequences of an untreated sample.
5. The method of claim 1 wherein the test compound which elicits the toxicological response is a compound not known to induce a toxicological response.
6. An isolated and purified polynucleotide selected from the group consisting of SEQ ID NOs: 2, 5-11, 13, 16-20, 22, 23, 25, 28, 29, 31-34, 36-39, 41, 42, 45-50, 52, 54-56, 58, 60, and 61.
7. A method of using the polynucleotide of claim 6 as a polynucleotide probe, the method comprising:
  - a) combining the polynucleotide of claim 6 with a polynucleotide target under conditions to allow formation of a complex; and
  - b) detecting the complex, thereby identifying a polynucleotide target which forms a complex with the polynucleotide of claim 6.
8. The method of claim 7 wherein the polynucleotide target is selected from the group consisting of mRNA, cDNA genomic DNA, **peptide** nucleic acids and branched DNA.

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Utility  
MARKERS FOR PEROXISOMAL PROLIFERATORS

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ISSUED: December 12, 2000 (20001212)  
INVENTOR(s): Cunningham, Mary Jane, Sunnyvale, CA (California), US (United States of America)  
Zweiger, Gary B., San Mateo, CA (California), US (United States of America)  
Panzer, Scott R., Sunnyvale, CA (California), US (United States of America)  
Seilhamer, Jeffrey J., Los Altos Hills, CA (California), US (United States of America)  
ASSIGNEE(s): Incyte Pharmaceuticals, Inc., (A U.S. Company or Corporation), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 27511]  
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Schena, M., et al., Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes, Proc. Natl. Acad. Sci. USA, 93:10614-10619, (Oct. 1996).

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Kroger, H., et al., Protection from Acetaminophen-Induced Liver Damage by the Synergistic Action of Low Doses of the Poly(ADP-ribose) Polymerase-Inhibitor Nicotinamide and the Antioxidant N-Acetylcysteine or the Amino Acid L-Methionine, Gen. Pharmac., 28:2, 257-263, (1997).

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PRIMARY EXAMINER: Jones, W. Gary  
ASST. EXAMINER: Siew, Jeffrey  
ATTORNEY, AGENT, OR FIRM: Incyte Genomics, Inc.  
CLAIMS: 8  
EXEMPLARY CLAIM: 1  
ART UNIT: 166

ABSTRACT

The present invention relates to a composition comprising a plurality of polynucleotide targets. The composition can be used as hybridizable array elements in a **microarray**. The present invention also relates to methods for screening compounds and therapeutic treatments for toxicological responses.

What is claimed is:

1. A method for detecting a toxicological effect of a test compound associated with increased or decreased levels of a polynucleotide sequence in a sample comprising:
  - a) treating a sample with a toxin;
  - b) obtaining a polynucleotide sequence from the sample treated with the toxin;
  - c) contacting the polynucleotide sequence with an array comprising a combination of polynucleotides comprising SEQ ID NOS. 1-56 under conditions effective to form one or more hybridization complexes;
  - d) detecting the hybridization complexes, wherein the presence, absence or change in amount of the hybridization complex, as compared with the hybridization complexes formed by SEQ ID NOS:1-56 with polynucleotide sequence from an untreated sample, is indicative of a toxicological response to the toxin; then
  - e) measuring the level of polynucleotide sequences in a sample treated with a test compound using the method of steps (c) and (d); and
  - f) comparing the level detected in step (e) with the level detected in step (c) wherein an increase or decrease in the level detected in step (e) indicates a toxicological response to the test compound.
2. The method of claim 1, wherein the sample is a tissue selected from the group consisting of liver, kidney, brain, spleen, pancreas and lung.
3. The method of claim 1, wherein the toxin is selected from the group consisting of hypolipidemic drugs, n-alkylcarboxylic acids, n-alkylcarboxylic acid precursors, azole antifungal compounds, leukotriene D4 antagonists, herbicides, pesticides, phthalate esters, phenyl acetate, dehydroepiandrosterone sulfate, oleic acid, methanol and their corresponding metabolites.
4. The method of claim 1, wherein the test compound which elicits the toxicological response induces at least a 2.5 fold change in the amount of the hybridization complexes formed between the polynucleotide of the array with at least one of the polynucleotide sequences of the sample, when compared with polynucleotide sequences of an untreated sample.
5. The method of claim 1 wherein the test compound which elicits the toxicological response is a compound not known to induce a toxicological response.
6. An isolated and purified polynucleotide selected from the group consisting of SEQ ID NOS:4-6, 8-10, 18-20, 23, 25, 27-30 32, 33, 36-39, 41-47, 49, and 51.
7. A method of using a polynucleotide as a polynucleotide probe, the method comprising:
  - a) combining the polynucleotide of claim 6 with a polynucleotide target under conditions to allow formation of a complex; and
  - b) detecting the complex, thereby identifying a polynucleotide target, which forms a complex with the polynucleotide of claim 6.

8. The method of claim 7 wherein the polynucleotide target is selected from the group consisting of mRNA, cDNA, genomic DNA, peptide nucleic acids, and branched DNA.

1/5/4 (Item 4 from file: 654)

DIALOG(R) File 654:US Pat.Full.

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03212493

Utility

METHOD AND APPARATUS FOR INTERPRETING HYBRIDIZED BIOELECTRONIC DNA MICROARRAY PATTERNS USING SELF-SCALING CONVERGENT REVERBERANT DYNAMICS

PATENT NO.: 6,142,681

ISSUED: November 07, 2000 (20001107)

INVENTOR(s): Gulati, Sandeep, La Canada, CA (California), US (United States of America)

ASSIGNEE(s): Vialogy Corporation, (A U.S. Company or Corporation), Altadena, CA (California), US (United States of America)

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U.S. CLASS: 395-13 cross ref: 435-6; 435-71.1; 435-91.1; 435-173;  
435-287.2; 435-288.7; 435-291; 436-173; 536-25.3; 536-25.4;  
935-77; 935-78

INTL CLASS: [7] G06F 15-18

FIELD OF SEARCH: 435-6; 435-71.1; 435-287.2; 435-288.7; 435-173; 435-91.1;  
435-291; 536-25.3; 536-25.4; 934-77; 934-78; 436-173

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PRIMARY EXAMINER: Horlick, Kenneth R.

ASST. EXAMINER: Taylor, Janell E.

ATTORNEY, AGENT, OR FIRM: Pretty, Schroeder & Poplawski, P.C.

CLAIMS: 31

EXEMPLARY CLAIM: 1

DRAWING PAGES: 6

DRAWING FIGURES: 4

ART UNIT: 163

#### ABSTRACT

A technique is described for identifying mutations, if any, present in a biological sample, from a pre-selected set of known mutations. The method can be applied to DNA, RNA and peptide nucleic acid (PNA) microarrays. The method analyzes a dot spectrogram representative of quantized hybridization activity of oligonucleotides in the sample to identify the mutations. In accordance with the method, a resonance pattern is generated which is representative of nonlinear resonances between a stimulus pattern associated with the set of known mutations and the dot spectrogram. The

resonance pattern is interpreted to yield a set of confirmed mutations by comparing resonances found therein with predetermined resonances expected for the selected set of mutations. In a particular example, the resonance pattern is generated by iteratively processing the dot spectrogram by performing a convergent reverberation to yield a resonance pattern representative of resonances between a predetermined set of selected Quantum Expressor Functions and the dot spectrogram until a predetermined degree of convergence is achieved between the resonances found in the resonance pattern and resonances expected for the set of mutations. The resonance pattern is analyzed to yield a set of confirmed mutations by mapping the confirmed mutations to known diseases associated with the pre-selected set of known mutations to identify diseases, if any, indicated by the biological sample. By exploiting a resonant interaction, mutation signatures may be robustly identified even in circumstances involving low signal to noise ratios or, in some cases, negative signal to noise ratios. What is claimed is:

1. A method for analyzing an output pattern of a biochip to identify mutations, if any, present in a biological sample applied to the biochip, said method comprising the steps of:
  - tessellating the output pattern;
  - generating a stimulus pattern associated with the set of known mutations;
  - generating a resonance pattern representative of resonances between the stimulus pattern and the tessellated output pattern;
  - interpreting the resonance pattern to yield a set of confirmed mutations by comparing resonances found therein with predetermined resonances expected for the selected set of mutations.
2. The method of claim 1 wherein the output pattern is derived from a biochip microarray having a plurality of cells each having a set of substantially identical immobilized oligonucleotides with the oligonucleotides of each cell being substantially unique from the oligonucleotides of every other cell.
3. The method of claim 1 wherein said step of tessellating the output pattern comprises the step of amplifying highly local morphological variations in the output pattern.
4. The method of claim 3 wherein the stimulus pattern is generated based upon Quantum Expressor Functions and wherein said step of tessellating the output pattern is performed to tessellate the output pattern to match morphological characteristic of the Quantum Expressor Functions.
5. The method of claim 1 further including the steps of:
  - extracting local parametrics from the tessellated output pattern;
  - determining whether a degree of amplitude wandering representative of the local parametrics is within a predetermined allowable generator function limit; and
  - if not, renormalizing the tessellated output pattern to further match spectral properties of the resonance pattern.
6. The method of claim 5 wherein said step of extracting local parametrics from the tessellated output pattern comprises the steps of
  - extracting parameters representative of an integrated density of states within each of a plurality tessellation regions within the tessellated output pattern.
7. The method of claim 5 wherein a degree of amplitude wandering is determined by applying a Palm distribution to develop generators for estimating stochastic wandering.
8. The method of claim 1 further including the step of transforming the tessellated output pattern and the stimulus pattern to a metrically transitive random field.
9. The method of claim 8 further including the step of renormalizing the tessellated output pattern following transformation to the metrically transitive random field.
10. The method of claim 9 wherein said step of renormalizing is performed



to rescale the tessellated output pattern to the interval.

11. The method of claim 1 wherein the step of generating a stimulus pattern associated with the set of known mutations comprises the steps of:  
selecting a sub-set of the mutations for analysis; and  
selecting a sub-set of nonlinear Quantum Expressor Functions from a set of predetermined nonlinear Quantum Expressor Functions based upon the selected sub-set of mutations.

12. The method of claim 11 further including the steps of:  
transforming the nonlinear Quantum Expressor Functions and the tessellated output pattern into phase space.

13. The method of claim 1 wherein the step of generating a resonance pattern includes the step of  
iteratively processing the output pattern by performing a convergent reverberation to yield a resonance pattern representative of resonances between a predetermined set of selected Quantum Expressor Functions and the tessellated output pattern until a predetermined degree of convergence is achieved between the resonances found in the resonance pattern and resonances expected for the set of mutations.

14. The method of claim 13 wherein the step of iteratively processing the output pattern by performing a convergent reverberation includes the step of performing a convergent reverberant dynamics resonance analysis of the tessellated output pattern using the resonance stimulus pattern to identify mutations represented by the tessellated output pattern.

15. The method of claim 14 wherein the step of performing a convergent reverberant dynamics resonance analysis includes the steps of:

- a) determining resonance dynamics relaxation values based upon the preconditioned output pattern and the resonance stimulus;
- b) filtering the dynamics relaxation values using ensemble boundary and CSR filters to yield a second set of values;
- c) applying bulk property estimators to the dynamics relaxation values to yield a third set of values;
- d) evaluating the second and third sets of values to determine a degree of resonance convergence; and
- e) determining from the degree of resonance convergence whether a paralysis of dynamics has occurred and, if so, repeating steps a)-e).

16. The method of claim 15 wherein said step of determining resonance dynamics relaxation values based upon the preconditioned output pattern and the resonance stimulus comprises the steps of  
applying a convolutionless evolution operator to the tessellated output pattern.

17. The method of claim 15 wherein said step of applying bulk property estimators to the dynamics relaxation values to yield a third set of values comprises the steps of

applying a coupling operator to which couples the dynamics relaxation values representative of the tessellated output pattern to a nonlinear information filter.

18. The method of claim 15 wherein said step of evaluating the second and third sets of values to determine a degree of resonance convergence comprises the steps of

determining whether the third set of values oscillate beyond and predetermined threshold after a predetermined period of time and, if not, convergence has been achieved.

19. The method of claim 18 wherein if no convergence has been achieved, performing the additional step of increasing a timescale for determining convergence beyond the predetermined period of time and, if convergence is still not achieved, then generating a signal indicating that none of the mutations of the mutation set are present in the sample.

20. The method of claim 15 wherein said step of determining whether a paralysis of dynamics as occurred comprises the steps of evaluating a Lindblad condition and, if the Lindblad condition has not been achieved

generating a signal indicating that paralysis of dynamics has occurred.

21. The method of claim 20 wherein, if a paralysis of dynamics has occurred performing the additional steps of determining whether a "mutation death" has occurred by varying a time scale for realization of the Lindbald condition and repeating the steps beginning with determining resonance dynamics relaxation values.

22. The method of claim 13 wherein the step of iteratively processing the output pattern by performing a convergent reverberation also includes the step of performing a convergent reverberant dynamics resonance analysis of the mutations using the resonance stimulus pattern to identify diagnostic conditions represented by the mutation.

23. The method of claim 22 wherein the step of performing a convergent reverberant dynamics resonance analysis of the mutations using the resonance stimulus pattern to identify diagnostic conditions represented by the mutations includes the steps of:

- a) determining resonance dynamics relaxation values based upon the resonance stimulus and the mutations;
- b) filtering the dynamics relaxation values using ensemble boundary and CSR filters to yield a second set of values;
- c) applying bulk property estimators to the dynamics relaxation values to yield a third set of values;
- d) evaluating the second and third sets of values to determine whether a predetermined degree of resonance convergence has been achieved; and
- e) if no convergence, repeat steps a)-e).

24. The method of claim 1 further including the step of filtering the diagnostic conditions identified by the convergent reverberant dynamics resonance analysis based upon clustering properties.

25. The method of claim 1 wherein the biological sample is selected from a group consisting of a DNA, RNA, protein, peptide-nucleic acid (PNA) and targeted nucleic amplification (TNA) samples.

26. The method of claim 1 further including the step of rendering a diagnostic decision based on the diagnostic conditions identified by the convergent reverberant dynamics resonance analysis.

27. The method of claim 26 wherein  
if said diagnostic decision is negative, then determining whether any alternatives are available; and  
if no alternatives are available, selecting a new sub-set of mutations and repeating all steps.

28. A method for preconditioning an output pattern of a biochip, said method comprising the steps of:

- a) tessellating the output pattern to match characteristics of a predetermined stimulus pattern yielding a tessellated output pattern;
- b) extracting local parametrics from the tessellated output pattern;
- c) determining whether a degree of amplitude wandering representative of the local parametrics is within a predetermined allowable generator function limit; and
- d) if not, renormalizing the tessellated output pattern to further match spectral properties of the stimulus pattern and repeat the steps b)-d).

29. A method for performing a convergent reverberant dynamics resonance analysis of a biochip output pattern to identify mutations represented thereby, said method comprising the steps of:

- a) determining resonance dynamics relaxation values based upon the preconditioned output pattern and the resonance stimulus;
- b) filtering the dynamics relaxation values using ensemble boundary and CSR filters to yield a second set of values;
- c) applying bulk property estimators to the dynamics relaxation values to yield a third set of values;
- d) evaluating the second and third sets of values to determine whether a predetermined degree of resonance convergence has been achieved; and
- e) determining whether a paralysis of dynamics has occurred and, if so,

repeat steps a)-e).

30. A system for analyzing an output pattern of a biochip to identify mutations, if any, present in a biological sample applied to the biochip, said system comprising:

- a tessellation unit operative to tessellate the output pattern;
- a stimulus pattern generation unit operative to generate a stimulus pattern associated with the set of known mutations;
- a resonance pattern generation unit operative to generate a resonance pattern representative of resonances between the stimulus pattern and the tessellated output pattern;
- a resonance pattern interpretation unit operative to interpret the resonance pattern to yield a set of confirmed mutations by comparing resonances found therein with predetermined resonances expected for the selected set of mutations.

31. A system for performing a convergent reverberant dynamics resonance analysis of a biochip output pattern to identify mutations represented thereby, said system comprising:

- a determination unit operative to determine resonance dynamics relaxation values based upon the preconditioned output pattern and the resonance stimulus;
- a filter operative to filter the dynamics relaxation values using ensemble boundary and CSR filters to yield a second set of values;
- a estimator unit operative to apply bulk property estimators to the dynamics relaxation values to yield a third set of values;
- an evaluation unit operative to evaluate the second and third sets of values to determine whether a predetermined degree of resonance convergence has been achieved; and
- a paralysis detection unit operative to detect whether a paralysis of dynamics has occurred.

1/5/5 (Item 5 from file: 654)

DIALOG(R) File 654:US Pat.Full.

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03205493

Utility

METHOD AND APPARATUS FOR ANALYZING HYBRIDIZED BIOCHIP PATTERNS USING RESONANCE INTERACTIONS EMPLOYING QUANTUM EXPRESSOR FUNCTIONS

PATENT NO.: 6,136,541

ISSUED: October 24, 2000 (20001024)

INVENTOR(s): Gulati, Sandeep, La Canada, CA (California), US (United States of America)

ASSIGNEE(s): Vialogy Corporation, (A U.S. Company or Corporation), Altadena, CA (California), US (United States of America)

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INTL CLASS: [7] C12Q 1-68; C07H 21-04; G06K 9-00

FIELD OF SEARCH: 435-6; 536-24.3; 536-24.31; 536-24.32; 364-130; 364-554; 364-578; 382-129

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PRIMARY EXAMINER: Horlick, Kenneth R.

ASST. EXAMINER: Siew, Jeffrey

ATTORNEY, AGENT, OR FIRM: Pretty, Schroeder & Poplawski, P.C.

CLAIMS: 31

EXEMPLARY CLAIM: 1

DRAWING PAGES: 6

DRAWING FIGURES: 7

ART UNIT: 166

#### ABSTRACT

A technique is described for identifying mutations, if any, present in a **biological** sample, from a pre-selected set of known mutations. The method can be applied to DNA, RNA and **peptide** nucleic acid (PNA) **microarrays**. The method analyzes a dot spectrogram representative of quantized hybridization activity of oligonucleotides in the sample to identify the mutations. In accordance with the method, a resonance pattern is generated which is representative of nonlinear resonances between a stimulus pattern associated with the set of known mutations and the dot spectrogram. The resonance pattern is interpreted to a yield a set of confirmed mutations by comparing resonances found therein with predetermined resonances expected for the selected set of mutations. In a particular example, the resonance pattern is generated by iteratively processing the dot spectrogram by performing a convergent reverberation to yield a resonance pattern representative of resonances between a predetermined set of selected Quantum Expressor Functions and the dot spectrogram until a predetermined degree of convergence is achieved between the resonances found in the resonance pattern and resonances expected for the set of mutations. The resonance pattern is analyzed to a yield a set of confirmed mutations by mapping the confirmed mutations to known diseases associated with the pre-selected set of known mutations to identify diseases, if any, indicated by the **biological** sample. By exploiting a resonant interaction, mutation signatures may be robustly identified even in circumstances involving low signal to noise ratios or, in some cases, negative signal to noise ratios. What is claimed is:

1. A method for analyzing an output pattern of a biochip to identify mutations, if any, present in a **biological** sample applied to the biochip, said method comprising the steps of:  
generating a resonance pattern representative of resonances between a stimulus pattern associated with a set of known mutations and the output pattern; and  
interpreting the resonance pattern to yield a set of confirmed mutations by comparing resonances found therein with predetermined resonances expected for the set of mutations.

2. The method of claim 1 wherein the output pattern is derived from a biochip having a plurality of cells each having a set of immobilized oligonucleotides with the oligonucleotides of each cell being different from the oligonucleotides of every other cell.

3. The method of claim 1 wherein the step of generating a resonance pattern includes the step of iteratively processing the output pattern by performing a convergent reverberation to yield a resonance pattern representative of resonances between a predetermined set of selected Quantum Expressor Functions and the output pattern until a predetermined degree of convergence is achieved between the resonances found in the resonance pattern and resonances expected for the set of mutations.

4. The method of claim 2 further including the steps of:  
selecting a sub-set of the mutations for analysis;  
generating preconditioner transforms based upon the selected sub-set of mutations by inverse Dirichlet tessellation on the biochip structure; and  
selecting a sub-set of nonlinear Quantum Expressor Functions from a set of predetermined nonlinear Quantum Expressor Functions based upon the selected sub-set of mutations.

5. The method of claim 1 further including the step of modifying the output pattern to generate a modified output pattern by performing one or more of the steps of:  
refocusing the output pattern to yield a re-focused output pattern;  
cross-correlating the re-focused output pattern;  
applying a local maxima filter to the correlated re-focused output pattern to yield a maximized output pattern;  
re-scaling the maximized output pattern to yield a uniformly re-scaled output pattern; and  
re-scaling the uniformly re-scaled output pattern to amplify local boundaries therein to yielding a globally re-scaled output pattern.

6. The method of claim 5 wherein the step of re-scaling the maximized output pattern is performed by applying an operator to selectively enhance those hixels and ensemble boundaries whose intensity exceeds a local average by more than a prespecified number of standard deviations.

7. The method of claim 5 wherein the step of resealing the uniformly re-scaled output pattern is performed by applying a logarithmic rescaling function around zero mean amplitudes within the output pattern to amplify local edge boundaries of the output pattern.

8. The method of claim 1 further including the step of estimating amplitude wanderings within the globally re-scaled output pattern.

9. The method of claim 8 wherein the step of estimating amplitude wanderings is performed by applying spectrogram ensemble invariance property generators constructed using a Palm distribution.

10. The method of claim 1 wherein the step of interpreting the resonance pattern to a yield a set of confirmed mutations by comparing resonances found therein with predetermined resonances expected for the set of mutations includes the step of  
mapping the confirmed mutations to known diseases associated with the pre-selected set of known mutations to identify diseases, if any, indicated by the biological sample.

11. The method of claim 1 further including the step of performing a diagnostic confirmation by taking the identified diseases and solving in reverse for the associated Quantum Expressor Functions and then comparing those Quantum Expressor Functions with ones expected for the mutations associated with the identified disease to verify correspondence and, if no correspondence is found, selecting a new sub-set of known mutations and repeating all steps.

12. The method of step 1 further including the initial steps of generating the set of nonlinear Quantum Expressor Functions by:  
inputting a set of mutation signatures representative of the pre-selected set of known mutations;  
inputting a representation of a biochip oligonucleotide pattern layout for the biochip from which the output pattern is generated;

generating a set of resonant interaction parameters representative of mutation pattern interactions between elements of the biochip including interactions from a group including element-to-element interactions, element-to-ensemble interactions, ensemble-to-element interactions, and ensemble-to-ensemble interactions; and generating the set of nonlinear Quantum Expressor Functions from the set of resonant interaction patterns.

13. The method of claim 12 wherein the step of generating the set of nonlinear Quantum Expressor Functions comprises the steps of matching a power spectral density (PSD) amplitude of a coded mutation signature, corresponding to the pre-selected mutation set of interest, to that of a Hamiltonian system so that stochastic and deterministic time scales match, and the time scales couple back to noise statistics and degree of asymmetry.

14. The method of step 1 further including the initial steps of generating the set of nonlinear Quantum Expressor Functions by: calculating values representative of a pre-selected Hamiltonian function; calculating harmonic amplitudes for the Hamiltonian function; generating an order function from the Hamiltonian; function measuring entrainment states of the order function; and modulating the order function using the entrainment states to yield the Quantum Expressor Function.

15. The method of step 14 wherein the Hamiltonian is a Spin-Boson Hamiltonian.

16. The method of claim 14 wherein the step of generating the order function includes the step of approximating a Daido Integral.

17. The method of claim 1 wherein the output pattern is a quantized output pattern.

18. The method of claim 17 wherein the quantized output pattern is a dot spectrogram.

19. The method of claim 1 wherein the biological sample selected from a group consisting of a DNA, RNA, protein, peptide-nucleic acid (PNA) and targeted nucleic amplification (TNA) samples.

20. A method for analyzing a biological sample to identify mutations, if any, present in the sample from a pre-selected set of known mutations, said method comprising the steps of:  
applying the sample to a biochip to generate an output pattern representative of quantized hybridization activity of oligonucleotides in the sample;  
selecting a sub-set of the mutations for analysis;  
generating preconditioner transforms based upon the selected sub-set of mutations;  
selecting a sub-set of nonlinear Quantum Expressor Functions from a set of predetermined nonlinear Quantum Expressor Functions based upon the selected sub-set of mutations;  
preconditioning the output pattern in accordance with the preconditioner transforms to generate a modified output pattern;  
iteratively processing the modified output pattern by performing a convergent reverberation to yield a resonance pattern representative of resonances between the selected set of Quantum Expressor Functions and the modified output pattern until a pre-selected degree of convergence is achieved between resonances of the resonance pattern and mutations of the selected sub-set of mutations; and  
interpreting the resonance pattern to yield a set of confirmed mutations by comparing the resonances therein with resonances expected for the mutations of the selected set of mutations.

21. The method of claim 20 further including the step of mapping the confirmed mutations to known diseases associated with the pre-selected set of known mutations to identify diseases, if any, indicated by the biological sample.

22. A system for analyzing an output pattern of a biochip to identify mutations, if any, present in a **biological** sample applied to the biochip, said system comprising:

resonance pattern for generating a resonance pattern representative of resonances between a stimulus pattern associated with a set of known mutations and the output pattern; and

a resonance pattern interpretation unit for interpreting the resonance pattern to yield a set of confirmed mutations by comparing resonances found therein with predetermined resonances expected for the selected set of mutations.

23. The system of claim 22 wherein the output pattern is derived from a biochip having a plurality of cells each having a set of immobilized oligonucleotides with the oligonucleotides of each cell being different from the oligonucleotides of every other cell.

24. The system of claim 22 wherein resonance pattern generator includes a resonant interaction unit for iteratively processing the output pattern by performing a convergent reverberation to yield a resonance pattern representative of resonances between a predetermined set of selected Quantum Expressor Functions and the output pattern until a predetermined degree of convergence is achieved between the resonances found in the resonance pattern and resonances expected for the set of mutations.

25. The system of claim 22 further including an image preconditioning unit for performing one or more of the functions of:

refocusing the output pattern to yield a re-focused output pattern;

cross-correlating the re-focused output pattern;

applying a local maxima filter to the correlated re-focused output pattern to yield a maximized output pattern;

re-scaling the maximized output pattern to yield a uniformly re-scaled output pattern; and

re-scaling the uniformly re-scaled output pattern to amplify local boundaries therein to yield a globally re-scaled output pattern.

26. The system of claim 22 wherein the resonance pattern interpretation unit for mapping the confirmed mutations to known diseases associated with the pre-selected set of known mutations to identify diseases, if any, indicated by the **biological** sample.

27. The system of step 22 further including a Quantum Expressor Function generator to:

input a set of mutation signatures representative of the set of known mutations;

input a representation of a biochip microarray pattern layout for the biochip from which the output pattern is generated;

generate a set of resonant interaction parameters representative of mutation pattern interactions between elements of the biochip including interactions from a group including element-to-element interactions, element-to-ensemble interactions, ensemble-to-element interactions, and ensemble-to-ensemble interactions; and

generate the set of nonlinear Quantum Expressor Functions from the set of resonant interaction patterns.

28. The system of claim 22 wherein the output pattern is a quantized output pattern.

29. The system of claim 28 wherein the quantized output pattern is a dot spectrogram.

30. The system of claim 28 wherein the **biological** sample is selected from a group consisting of a DNA, RNA, **protein**, **peptide**-nucleic acid (PNA) and targeted nucleic amplification (TNA) samples.

31. A system of generating a set of nonlinear Quantum Expressor Functions based upon a set of mutation signatures representative of a set of known mutations and based upon a biochip microarray pattern, said system comprising:

a resonant interaction parameter generator for generating a set of

resonant interaction parameters representative of mutation pattern interactions between elements of the biochip including interactions selected from the group consisting of element-to-element interactions, element-to-ensemble interactions, ensemble-to-element interactions, and ensemble-to-ensemble interactions; and a quantum expressor functions generator for generating a set of nonlinear Quantum Expressor Functions from the set of resonant interaction patterns.

1/5/6 (Item 6 from file: 654)  
DIALOG(R) File 654:US Pat.Full.  
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03176840

Utility

METHODS FOR FABRICATING MICROARRAYS OF BIOLOGICAL SAMPLES

PATENT NO.: 6,110,426  
ISSUED: August 29, 2000 (20000829)  
INVENTOR(s): Shalon, Tidhar Dari, Atherton, CA (California), US (United States of America)  
Brown, Patrick O., Stanford, CA (California), US (United States of America)  
ASSIGNEE(s): The Board of Trustees of the Leland Stanford Junior University, (A U.S. Company or Corporation), Stanford, CA (California), US (United States of America)  
[Assignee Code(s): 49136]  
APPL. NO.: 9-1,027  
FILED: December 30, 1997 (19971230)

#### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 08-477,809, filed Jun. 7, 1995 and now U.S. Pat. No. 5,807,522, which is a continuation-in-part of U.S. patent application Ser. No. 08-261,388, filed Jun. 17, 1994 and now abandoned, both of which are incorporated herein by reference.

The United States government may have certain rights in the present invention pursuant to Grant No. HG00450 awarded by the National Institutes of Health.

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INTL CLASS: [7] G01N 15-06  
FIELD OF SEARCH: 422-50; 422-68.1; 435-6; 435-183.1; 436-501; 935-77; 935-78; 536-25.3

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PRIMARY EXAMINER: Marschel, Ardin H.  
ATTORNEY, AGENT, OR FIRM: Arnold White & Durkee

CLAIMS: 4  
EXEMPLARY CLAIM: 1  
DRAWING PAGES: 6  
DRAWING FIGURES: 14  
ART UNIT: 165

#### ABSTRACT

A method and apparatus for forming microarrays of biological samples on a support are disclosed. The method involves dispensing a known volume of a reagent at each selected array position, by tapping a capillary dispenser on the support under conditions effective to draw a defined volume of liquid onto the support. The apparatus is designed to produce a microarray of such regions in an automated fashion.

It is claimed:

1. A method of forming a microarray of discrete analyte-assay regions on a solid support, where each discrete region in the microarray has a select(d, analyte-specific reagent, said method comprising,
  - (a) loading a solution of a selected analyte-specific reagent in a reagent-dispensing device having an elongate capillary channel adapted to hold a quantity of the reagent solution and having a tip region at which the channel is open-sided and the solution in the channel forms a meniscus,
  - (b) tapping the tip of the dispensing device against a solid support at a defined position on the surface, with an impulse effective to break the meniscus in the capillary channel and deposit a selected volume of solution on the surface, and
  - (c) repeating steps (a) and (b) until said microarray is formed.
2. The method of claim 1, wherein the reagents used to form the discrete regions in the microarray are distinct nucleic acid strands and wherein steps (a) and (b) are repeated until the microarray has about 100 or more discrete regions of distinct nucleic acid strands per cm sup 2 of solid support.
3. The method of claim 1, wherein the reagents used to form the discrete regions in the microarray are distinct nucleic acid strands and wherein steps (a) and (b) are repeated until the microarray has about 1000 or more discrete regions of distinct nucleic acid strands per cm sup 2 of solid support.
4. The method of claim 1, which further includes, after performing steps (a) and (b), reloading the reagent-dispensing device with a new reagent solution by the steps of (i) dipping the capillary channel of the device in a wash solution, (ii) removing wash solution drawn into the capillary channel, and (iii) dipping the capillary channel into the new reagent solution.

1/5/7 (Item 7 from file: 654)  
DIALOG(R) File 654:US Pat.Full.  
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03167518

Utility

**MICROARRAY** PRINTING DEVICE INCLUDING PRINTING PINS WITH FLAT TIPS AND EXTERIOR CHANNEL AND METHOD OF MANUFACTURE

PATENT NO.: 6,101,946  
ISSUED: August 15, 2000 (20000815)  
INVENTOR(s): Martinsky, Richard S, San Jose, CA (California), US (United States of America)  
ASSIGNEE(s): TeleChem International Inc , (A U.S. Company or Corporation), Sunnyvale, CA (California), US (United States of America)  
APPL. NO.: 9-191,935  
FILED: November 13, 1998 (19981113)

RELATED APPLICATIONS

This is a continuation of Provisional patent 70014 U.S. PTO 60-066,733 filed on Nov. 21, 1997 with the title "ChipMaker".

U.S. CLASS: 101-494 cross ref: 222-420; 422-50; 422-100; 422-920;  
435-283.1

INTL CLASS: [7] B01L 3-02

FIELD OF SEARCH: 101-494; 400-118.2; 422-50; 422-920; 422-100; 435-283.1;  
222-420

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PRIMARY EXAMINER: Hilten, John S.

ASST. EXAMINER: Colilla, Dan

ATTORNEY, AGENT, OR FIRM: Skjerven, Morrill, MacPherson, Franklin and Friel

CLAIMS: 10

EXEMPLARY CLAIM: 1

DRAWING PAGES: 9

DRAWING FIGURES: 15

ART UNIT: 284

#### ABSTRACT

The invention herein describes a device for fabricating **microarrays** of biochemical substances, consisting of a holder and one or more printing pins. The holder contains apertures with regular spacing that define the location of one or more printing pins during the printing process. The tip of each printing pin contains a sample channel that holds a predetermined volume of **biological** or chemical sample and a point that is machined to precision with an electronic discharge machine (EDM). The device can be attached to a motion control system for precise and automated movement in three dimensions. The flat tips of the pins are immersed in a biochemical sample such that a predefined volume of sample fills the sample channel of each pin. The holder and pins are then moved in proximity to a printing substrate whereby direct contact between the flat tips of the pins and the surface results in the transfer of a small amount of the sample onto the solid surface. The holder and pins are mass produced at high precision to ensure that the printed elements in the resultant **microarray** contains approximately the same quantity of sample. In one preferred embodiment, the device is employed to manufacture arrays of nucleic acids or derivatives thereof.

What is claimed is:

1. A device for printing microarrays comprising:  
a holder; and  
one or more printing pins that are mounted in said holder, each of said one or more printing pins having an exterior sample channel for holding a predetermined volume of a sample and a flat tip.
2. The device of claim 1 wherein said holder accommodates up to 32 pins.
3. The device of claim 1 wherein each of said one or more printing pins has a collar to prevent rotation of said one or more printing pins in said holder.
4. The device of claim 3 wherein said one or more printing pins is movably mounted in said holder, said one or more printing pins being movable in a vertical direction, said one or more printing pins having a rest position wherein a bottom surface of said collar contacts said holder stopping the downward movement of said one or more printing pins.
5. A method of making a printing pin comprising:  
providing a pin shaft;  
producing a point having a flat tip at a first end of said pin shaft; and  
producing an exterior sample channel at said point of said pin shaft, thereby creating a gap at said point.

6. The method of claim 5 further comprising:  
forming a collar; and  
attaching said collar to the second end of said pin shaft.
7. The method of claim 5 further comprising adjusting the width of said gap at said point.
8. The method of claim 5 wherein said producing a point comprises forming four angular sides on said first end of said pin shaft, said four angular sides having an apex that forms a square flat tip in the approximate geometric center of said pin shaft.
9. The method of claim 8 wherein said forming four angular sides comprises cutting with an electronic discharge machine (EDM).
10. The method of claim 5 wherein said producing an exterior sample channel comprises cutting with an electronic discharge machine (EDM).

1/5/8. (Item 8 from file: 654)  
DIALOG(R) File 654:US Pat.Full.  
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03107320

#### Utility

#### CHEMICALLY MODIFIED NUCLEIC ACIDS AND METHODS FOR COUPLING NUCLEIC ACIDS TO SOLID SUPPORT

[Nucleic acid modified by addition of epoxide-alkoxysilane compound for immobilization to unmodified or underivatized glass surface; for hybridization microarrays for genome-wide genetic mapping and gene expression studies]

PATENT NO.: 6,048,695  
ISSUED: April 11, 2000 (20000411)  
INVENTOR(s): Bradley, Allan, Houston, TX (Texas), US (United States of America)  
Cai, Wei Wen, Houston, TX (Texas), US (United States of America)  
ASSIGNEE(s): Baylor College of Medicine, (A U.S. Company or Corporation), Houston, TX (Texas), US (United States of America)  
[Assignee Code(s): 6345]  
APPL. NO.: 9-71,876  
FILED: May 04, 1998 (19980504)  
U.S. CLASS: 435-6 cross ref: 436-524; 536-26.6  
INTL CLASS: [7] C12Q 1-68  
FIELD OF SEARCH: 435-6; 436-524; 536-26.6

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5,807,756     9/1998     Bauman et al.

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436-524

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PRIMARY EXAMINER: Houtteman, Scott W.

ATTORNEY, AGENT, OR FIRM: Fulbright & Jaworski, L.L.P.

CLAIMS: 51

EXEMPLARY CLAIM: 1

DRAWING PAGES: 6

DRAWING FIGURES: 9

ART UNIT: 165

# ABSTRACT

The invention relates to novel chemically modified nucleic acids with enhanced lability towards solid supports, such as glass. These modified nucleic acids can be readily affixed to solid supports, for instance, a glass surface, without first derivatizing the glass surface. High-density microarrays based on these modified nucleic acids as well as methods for preparing these microarrays are also useful.

What is claimed is:

1. A modified nucleic acid comprising a nucleic acid covalently bound to a compound having the formula:  $R_{sub 1} - X - R_{sub 2}$  wherein  $R_{sub 1}$  is an epoxide group; wherein  $R_{sub 2}$  is an alkoxysilane group; and wherein  $X$  is moiety, chemically suitable for linking said epoxide group and said alkoxysilane group.
2. The modified nucleic acid of claim 1 wherein said epoxide group is ethylene oxide.
3. The modified nucleic acid of claim 1 wherein said alkoxysilane is selected from the group consisting of  $--Si(OCH_{sub 3})_{sub 3}$ ,  $--Si(OC_{sub 2}H_{sub 5})_{sub 3}$ ,  $--Si(OCH_{sub 3})_2H_{sub 2}$ ,  $--Si(OCH_{sub 3})(CH_{sub 3})_{sub 2}$ , and  $--Si(OCH_{sub 3})_{sub 2}CH_{sub 3}$ .
4. The modified nucleic acid of claim 1 wherein said compound is 3-glycidopropyltrimethoxysilane.
5. The modified nucleic acid of claim 1 wherein said nucleic acid is DNA.
6. The modified nucleic acid of claim 1 wherein said nucleic acid is RNA.
7. A modified nucleic acid comprising a nucleic acid covalently bound to a compound having the formula:  $R_{sub 1} - X - R_{sub 2}$  wherein  $R_{sub 1}$  is an amino group; wherein  $R_{sub 2}$  is an alkoxysilane group; and wherein  $X$  is moiety, chemically suitable for linking said amino group and said alkoxysilane group.
8. The modified nucleic acid of claim 7 wherein said amino group is a primary amine.
9. The modified nucleic acid of claim 7 wherein said alkoxysilane is selected from the group consisting of  $--Si(OCH_{sub 3})_{sub 3}$ ,  $--Si(OC_{sub 2}H_{sub 5})_{sub 3}$ , and [See structure in original document]3## wherein  $R_{sub 1}$ ,  $R_{sub 2}$ , and  $R_{sub 3}$  are selected from the group consisting of  $--H$ ,  $--CH_{sub 3}$ ,  $--OCH_{sub 3}$ , and  $--OC_{sub 2}H_{sub 5}$ , and provided that at least one of  $R_{sub 1}$ ,  $R_{sub 2}$ , or  $R_{sub 3}$  is either  $--OCH_{sub 3}$ , or  $--OC_{sub 2}H_{sub 5}$ .
10. The modified nucleic acid of claim 7 wherein said compound is 3-aminopropyltriethoxysilane.
11. The modified nucleic acid of claim 7 wherein said nucleic acid is DNA.
12. The modified nucleic acid of claim 7 wherein said nucleic acid is RNA.
13. The modified nucleic acid of claim 7 wherein said nucleic acid contains a cytosine residue.
14. A device for making high-density microarrays comprising a plurality of capillary micropipets, having a distal and a tapered proximal end, wherein said micropipets are:  
arranged equatorially in a substantially close-packed arrangement; and  
tapered at each said proximal end and wherein said ends are essentially planar.

15. The device of claim 14 wherein said micropipets are arranged in a close-packed square arrangement.

16. The device of claim 14 wherein said micropipets are bound by a frame.

17. The device of claim 16 wherein said micropipets are affixed to a base.

18. A high-density microarray comprising:  
a solid support;  
modified nucleic acid prepared by reacting said nucleic acid with an alkoxysilane selected from the group consisting of 3-glycidoxypropyltrimethoxysilane and 3-aminopropyltriethoxysilane, and immobilized about said solid support in orderly discrete spots.

19. The high-density microarray of claim 18 wherein said solid support is glass.

20. The high-density microarray of claim 18 wherein said discrete spots are about 50 microns in diameter.

21. A high-density microarray comprising:  
the modified nucleic acid of claim 18;  
a solid support upon which said plurality of closely spaced samples of said modified nucleic acid samples are placed.

22. The high-density microarray of claim 18 wherein said samples are placed upon said solid support using a device for making high-density microarrays.

23. A high-density microarray comprising a solid support having a plurality of modified silane moieties affixed, wherein said silane moieties are selected from the group consisting of --Si--(CH sub 2) sub n --, wherein n=3, 4, 5, 6, 7, 8, or 9; and --Si--CH sub 2 --CH sub 2 --C sub 6 H sub 4 --CH sub 2 --CH sub 2 --Si--.

24. A method for modulating the electrostatic properties of a glass solid support comprising selectively reacting said solid support with silanes selected from the group consisting of: 1,6-Bis-trichlorosilylhexane, 1,8-Bis-trichlorosilyloctane, 1,6-Bis-trimethoxysilylhexane, and 1,4-Bis-trimethoxysilyl ethylbenzene.

25. A modified nucleic acid prepared by:  
reacting a guanine or cytosine base of said nucleic acid with N-bromosuccinimide at pH about 8.0 to form a brominated nucleic acid;  
reacting said brominated nucleic acid with a silane having the formula H sub 2 N--(CH sub 2) sub n --Si(OC sub 2 H sub 5) sub 3, wherein n=3, 4, 5, 6, 7, 8, or 9.

26. A modified nucleic acid comprising a nucleic acid covalently bound to a compound having the formula:--HN--(CH sub 2) sub n --Si(OR) sub 3

wherein n=3, 4, 5, 6, 7, 8, or 9.

27. The modified nucleic acid of claim 26 wherein R is selected from the group consisting of --CH sub 3, --C sub 2 H sub 5, and C sub 3 H sub 7.

28. A modified nucleic acid comprising a nucleic acid covalently bonded to a compound having the formula: [See structure in original document]4## wherein R is selected from the group consisting of --CH sub 3, --C sub 2 H sub 5, and --C sub 3 H sub 7 ;

wherein R sub 1 and R sub 2 are the same or different and are selected from the group consisting of --H, --CH sub 3, --C sub 2 H sub 5, --OCH sub 3, --OC sub 2 H sub 5, --C sub 3 H sub 7, and --OC sub 3 H sub 7 ; and wherein X linking group comprising an at least partially aliphatic chain.

29. A method for immobilizing nucleic acid to solid support comprising:  
reacting a compound of the formula R sub 1 --X--R sub 2 with a nucleic acid to form a derivatized nucleic acid; wherein R sub 1 is an epoxide

group; wherein R sub 2 is an alkoxyasilane group; and, wherein X is a moiety chemically suitable for linking said epoxide group and said alkoxyasilane group; and,  
reacting said derivatized nucleic acid with said solid support.

30. The method of claim 29 wherein said compound is 3-glycidoxypropyltrimethoxysilane.

31. A method for immobilizing nucleic acid to solid support comprising:  
reacting compound of the formula R sub 1 --X--R sub 2 with a nucleic acid to form a derivatized nucleic acid; wherein R sub 1 is an amino group; wherein R sub 2 is an alkoxyasilane group; and, wherein X is a moiety chemically suitable for linking said amino group and said alkoxyasilane group; and  
reacting said derivatized nucleic acid with said solid support.

32. The method of claim 31 wherein said compound is 3-aminopropyltriethoxysilane.

33. A modified nucleic acid comprising a nucleic acid covalently bound to a compound having the formula: R sub 1 --X--R sub 2  
wherein R sub 1 is a cyclic ether;  
wherein R sub 2 is --NR sub 4, wherein each R sub 3 group are the same or different alkyl groups, and R sub 4 is --H or an alkyl group; and  
wherein X is moiety, chemically suitable for linking said cyclic ether group and said alkoxyasilane group.

34. A modified nucleic acid comprising a nucleic acid covalently boded to a compound having the formula: [See structure in original document]5##  
wherein R sub 1, R sub 2, and R sub 3, are the same or different, and are selected from the group consisting of --OCH sub 3, --OC sub 2 H sub 5, --OC sub 3 H sub 7, and --Cl; and  
wherein X is a moiety, chemically suitable for linking said nucleic acid to said compound.

35. The method of claim 29 or 30 wherein said nucleic acid is DNA.

36. The method of claim 29 or 30 wherein said nucleic acid is RNA.

37. The method of claim 29 or 30 wherein said first reacting step occurs at basic pH.

38. The method of claim 35 wherein said first reacting step occurs at pH from about 6 to about 12.

39. The method of claim 36 wherein said first reacting step occurs at pH from about 6 to about 8.5.

40. The method of claim 37 wherein said pH is greater than 9.5.

41. The method of claim 29 or 30 wherein said solid support is glass.

42. The method of claim 29 or 30 wherein said second reacting step occurs at approximately neutral pH.

43. The method of claim 31 or 32 wherein said nucleic acid is DNA.

44. The method of claim 31 or 32 wherein said nucleic acid is RNA.

45. The method of claim 31 or 32 wherein said nucleic acid contains a cytosine residue.

46. The method of claim 31 or 32 wherein said first reacting step occurs at essentially neutral pH.

47. The method of claim 44 wherein said first reacting step occurs at about pH=6.0-7 slashed zero

48. The method of claim 31 or 32 wherein said first reacting step occurs

in the presence of sodium bisulfite.

49. The method of claim 31 or 32 wherein said solid support is glass.

50. A kit for performing a hybridization assay on nucleic acid samples comprising:

a solid support; and  
a device for imprinting an array of DNA chips of modified nucleic acids of claim 1, 7, 25, 28, or 34 onto said solid support.

51. The modified nucleic acid of claim 33 wherein said compound selected from the group consisting of --(CH sub 2) sub 8 SiCl sub 3, --(CH sub 2) sub 8 Si(OCH sub 3) sub 3, --(CH sub 2) sub 4 SiCl sub 2 CH sub 3, and --CH sub 2 CH sub 2 CH sub 2 Si(OCH sub 3) sub 3.

1/5/9 (Item 9 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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03058236

Utility

QUANTITATIVE MICROARRAY HYBRIDIZATON ASSAYS  
[Quantitative gene expression analysis]

PATENT NO.: 6,004,755

ISSUED: December 21, 1999 (19991221)

INVENTOR(s): Wang, Bruce, Pacifica, CA (California), US (United States of America)

ASSIGNEE(s): Incyte Pharmaceuticals, Inc , (A U.S. Company or Corporation), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 27511]

APPL. NO.: 9-56,338

FILED: April 07, 1998 (19980407)

U.S. CLASS: 435-6 cross ref: 536-26.6

INTL CLASS: [6] C12Q 1-68

FIELD OF SEARCH: 435-6; 536-26.6; 536-24.3; 536-24.31; 536-24.32

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PRIMARY EXAMINER: Houtteman, Scott W.  
ATTORNEY, AGENT, OR FIRM: Bozicevic, Field & Francis LLP Field, Bret  
CLAIMS: 17  
EXEMPLARY CLAIM: 1  
DRAWING PAGES: 5  
DRAWING FIGURES: 7  
ART UNIT: 165

#### ABSTRACT

Methods are provided for quantitative gene expression analysis. In the subject methods, end-labeled target nucleic acid is contacted with an array of probe molecules stably associated with the surface of a solid support under hybridization conditions sufficient to produce a hybridization pattern. The resultant hybridization pattern can be used to obtain a quantitative information about the genetic profile of the end-labeled target nucleic acid sample, as well as the physiological source from which it is derived. As such, the subject methods find use in a variety of applications.

What is claimed is:

1. A hybridization assay comprising the steps of:  
contacting an array of probe molecules stably associated with the surface of a solid support with an end labeled target nucleic acid sample under hybridization conditions sufficient to produce a hybridization pattern, wherein each of said end labeled target nucleic acids is capable of generating a signal of substantially the same specific activity; and detecting said hybridization pattern.
2. The assay according to claim 1 further comprising the steps of removing unhybridized target nucleic acid prior to said detecting step.
3. The assay according to claim 1, wherein said method further comprises preparing said end labeled target nucleic acid sample by contacting an mRNA source with an end-labeled oligo(dT) primer, a reverse transcriptase and nucleotides under conditions sufficient for reverse transcription of said mRNA into said end-labeled target nucleic acid sample, wherein said end-labeled oligo(dT) primer comprises a known number of labeled nucleotides.
4. The assay according to claim 1, wherein said end-labeled nucleic acid sample further comprises end-labeled standard DNA.
5. The assay according to claim 4, wherein said assay further comprises:
  - (a) preparing said end-labeled standard DNA by contacting a standard RNA sample with said end-labeled oligo(dT) primer, a reverse transcriptase and nucleotides under conditions sufficient for reverse transcription of said standard RNA into said end labeled standard DNA; and
  - (b) combining said end labeled standard DNA with said end-labeled target nucleic acid sample.
6. The assay according to claim 1, wherein said array is contacted with a nuclease prior to said detecting step.
7. An assay to determine the genetic profile of a physiological source, said assay comprising the steps of:

(a) preparing an end labeled target nucleic acid sample by contacting mRNA from a physiological source with an end-labeled oligo(dT) primer, a reverse transcriptase and nucleotides under conditions sufficient for reverse transcription of said mRNA into said end-labeled target nucleic acid sample, wherein said end-labeled oligo (dT) primer is comprises a known number of labeled nucleotides;

(b) preparing end labeled standard DNA by contacting standard RNA with said end-labeled oligo(dT) primer, a reverse transcriptase and nucleotides under conditions sufficient for reverse transcription of said standard RNA into said end labeled standard DNA;

(c) combining said end labeled standard DNA with said end-labeled target nucleic acid sample;

(d) contacting said end labeled target nucleic acid sample with an array of probe molecules stably associated with the surface of a solid support under hybridization conditions to produce a hybridization pattern;

(e) separating unhybridized target from said array;

(f) contacting said array with a nuclease; and

(g) detecting said hybridization pattern.

8. The assay according to claim 7, wherein said end-labeled target nucleic acids are not directly detectable and said method further comprises contacting said hybridization pattern with at least one additional member of a signal producing system to provide a detectable hybridization pattern.

9. The assay according to claim 8, wherein said contacting with at least one additional member of a signal producing system is prior to said step (f).

10. The assay according to claim 8, wherein said end-labeled target nucleic acids are labeled with biotin.

11. The assay according to claim 9, wherein said at least one additional member of a signal producing system is fluorescently labeled streptavidin.

12. A kit for use in the preparation of end-labeled target nucleic acids to be used in an array based assay, said kit comprising:

end labeled oligo(dT) primers capable of generating a signal of known value; and a polymerase.

13. The kit according to claim 12, wherein said kit further comprises nucleotides.

14. The kit according to claim 12, wherein said kit further comprises a buffer.

15. The kit according to claim 12, wherein said kit further comprises an array of probe molecules and said kit is for use in a hybridization assay.

16. The kit according to claim 12, wherein said kit further comprises standard RNA.

17. The kit according to claim 12, wherein said kit further comprises a nuclease.

1/5/10 (Item 10 from file: 654)

DIALOG(R) File 654:US Pat.Full.

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02842487

Utility

METHODS FOR FABRICATING MICROARRAYS OF BIOLOGICAL SAMPLES

[ Tapping capillary dispenser on support with impulse to break meniscus; gene expression studies to diagnose disease]

PATENT NO.: 5,807,522

ISSUED: September 15, 1998 (19980915)

INVENTOR(s): Brown, Patrick O., Stanford, CA (California), US (United States of America)

Shalon, Tidhar Dari, Atherton, CA (California), US (United States of America)  
ASSIGNEE(s): The Board of Trustees of the Leland Stanford Junior University  
, (A U.S. Company or Corporation), Stanford, CA (California),  
US (United States of America)  
[Assignee Code(s): 49136]  
APPL. NO.: 8-477,809  
FILED: June 07, 1995 (19950607)

#### CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. patent application Ser. No. 08-261,388, filed Jun. 17, 1994, and now abandoned.

The United States government may have certain rights in the present invention pursuant to Grant No. HG00450 awarded by the National Institutes of Health.

U.S. CLASS: 422-50 cross ref: 422-52; 422-55; 422-56; 422-57; 422-68.1;  
422-69; 422-82.05; 422-82.06; 422-82.07; 422-82.08; 435-6;  
435-7.1; 436-501; 530-300; 530-333; 530-334; 530-350; 536-25.3  
INTL CLASS: [6] C12M 1-34; C12M 1-40  
FIELD OF SEARCH: 435-6; 435-7.1; 435-172.3; 536-23.1; 536-24.31; 536-25.3;  
935-78; 935-3; 935-19; 935-80; 436-501; 436-813; 422-50;  
422-52; 422-55; 422-56; 422-57; 422-68.1; 422-69; 422-82.5;  
422-82.6.08; 530-300; 530-333; 530-334; 530-350

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PRIMARY EXAMINER: Marschel, Ardin H.

ATTORNEY, AGENT, OR FIRM: Arnold White & Durkee

CLAIMS: 7

EXEMPLARY CLAIM: 1

DRAWING PAGES: 6

DRAWING FIGURES: 14

ART UNIT: 189

#### ABSTRACT

A method and apparatus for forming microarrays of biological samples on a support are disclosed. The method involves dispensing a known volume of a reagent at each selected array position, by tapping a capillary dispenser on the support under conditions effective to draw a defined volume of liquid onto the support. The apparatus is designed to produce a microarray of such regions in an automated fashion.

We claim:

1. A method of forming a microarray of discrete analyte-assay regions on a solid support, where each discrete region in the microarray has a selected, analyte-specific reagent, said method comprising,

(a) loading an aqueous solution of a selected analyte-specific reagent in a reagent-dispensing device having an elongate capillary channel adapted to hold a quantity of the reagent solution and having a tip region at which the solution in the channel forms a meniscus,

(b) tapping the tip of the dispensing device against a solid support at a defined position on the surface, with an impulse effective to break the meniscus in the capillary channel and deposit a selected volume between 0.002 and 2 nl of solution on the surface, and

(c) repeating steps (a) and (b) until said microarray is formed.

2. The method of claim 1, wherein the reagents used to form the discrete regions in the microarray are distinct nucleic acid strands and wherein steps (a) and (b) are repeated until the microarray has about 100 or more discrete regions of distinct nucleic acid strands per  $\text{cm}^2$  of solid support.

3. The method of claim 1, wherein the reagents used to form the discrete regions in the microarray are distinct nucleic acid strands and wherein steps (a) and (b) are repeated until the microarray has about 1000 or more discrete regions of distinct nucleic acid strands per  $\text{cm}^2$  of solid support.

4. The method of claim 2, wherein the channel is open-sided.

5. The method of claim 3, wherein the channel is open-sided.

6. The method of claim 4, wherein the volume is between 0.002 and 0.25 nl.

7. The method of claim 5, wherein the volume is between 0.002 and 0.25 nl.

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Responses to toxins show how adaptive changes can be based on major genes with polygenic modifiers. Analyses of continuous variation in ecologically relevant traits indicate low levels of heritable variation in some natural situations and highlight the importance of genetic interactions. The role of major and minor genes in adaptive responses in quantitative traits is unclear. Some evidence suggests that genetic trade-offs exist among environments and life history traits.

## DESCRIPTORS:

Population genetics; Quantitative genetics; Genetics and environment

*Record - 59*

DIALOG(R) File 112:UBM Industry News  
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01217809 (USE FORMAT 7 OR 9 FOR FULLTEXT)

*IFT EXPO - same game, different name*

Food Manufacture , p 46

September, 1999

LANGUAGE: English RECORD TYPE: Fulltext DOC. TYPE: Journal

WORD COUNT: 00002377

## SIC CODES/DESCRIPTORS:

2869 .(Industrial Organic Chemicals, NEC) - Company acquisition

2075 .(Soybean Oil Mills) - Company organisation

20 .(Food and Kindred Products) - Trade events

2099 .(Food Preparations, NEC) - Product information

2087 .(Flavoring Extracts and Syrups, NEC) - Product information

2046 .(Wet Corn Milling) - Product information

2869 .(Industrial Organic Chemicals, NEC) - Product information

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2086 .(Bottled and Canned Soft Drinks) - Product information

2066 .(Chocolate and Cocoa Products) - Product information

3556 .(Food Products Machinery) - Product information

204 .(Grain Mill Products) - Product information

COMPANY NAMES: Danisco-Cultor; McNeil Speciality Products; Cerestar; DSM Food Specialities; Monsanto; Lucas Meyer Group; SKW Trostberg; FMC; Pronova Biopolymer; Pronova; Avonmore Waterford Ingredients; Roche Vitamins; Watson; Folexco; National Starch and Chemical; Armfield; Chaucer Food Group; Kerry Inc

COUNTRY: Norway;

REGION: West Europe; USA

*Record - 60*

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*Pharmacogenomic Strategies: Biotech's New David and Goliath Challenge*

Bioventure-View 1304 pl, April 01, 1998 (19980401)

STORY TYPE: F WORD COUNT: 3477

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You've heard the buzzword. Lots of companies are talking about their move into pharmacogenomics, the analysis of genetic differences among patients that could mark them as better or worse candidates for a particular drug ... or lead to highly targeted, even "personalized" drugs.

If you can efficiently diagnose a patient in terms of DNA mutations, alleles, or polymorphisms pertaining to a specific disease, you'll potentially have vital information for improving their response to treatment.

Pharmacogenomics is an idea that has just been waiting for the technology to make it possible. The basic ingredients are nothing new. DNA-based diagnostics, for instance, have been sold for almost 20 years. Likewise, the desire to identify subgroups of a population most likely to benefit from drugs is an impulse with some history behind it.

For commercial pharmacogenomics to be practical, researchers need a method for high capacity, cost-effective, rapid DNA identification and a large amount of existing sequence data so patient genes can be compared to the "norm."

Both of these key areas have only recently reached critical mass. The initiation of the Human Genome Project in 1992 started the race to sequence the complete human genome. Many companies, including Human Genome Sciences Inc. (Rockville, MD), Incyte Pharmaceuticals Inc. (Palo Alto, CA), Millennium Pharmaceuticals Inc. (Cambridge, MA), Genset SA (Paris, France), AxyS Pharmaceuticals Inc. (South San Francisco, CA), and Hyseq Inc. (Sunnyvale, CA), have built up large databases of linear sequence information, and huge amounts of publicly available sequence data have also been amassed.

That's just the beginning, however. Having a sequence of the entire human genome - or the entire genomes of several individuals - tells you little about the genetic mutations and polymorphisms that may be implicated in a specific disease. That work still needs to be done, and has led to the birth of several "pure play" pharmacogenomic companies like Genaissance Pharmaceuticals Inc. (New Haven, CT) and Variagenics Inc. (Cambridge, MA), as well as a major initiative Hyseq and by Genset (in collaboration with Abbott Laboratories).

#### Lots of Work To Do

Behind these efforts are advances in technology that will allow small portions of the genome involved in a disease to be sequenced over and over again in thousands of people and matched against individuals' clinical information on symptoms, treatments, and outcomes.

Discovering mutants, alleles, polymorphisms - what Genaissance calls "isogenes" - is just the first step. "The key thing is that you don't just have to discover things, you have to validate them," says Dr. Gualberto Ruano, founder and CEO of Genaissance. Companies like his that are dedicated to the creation of personalized medicines are hoping they have the key technology that can make the leap from numerous DNA anomalies to a few key disease-related gene variants.

Once you've learned which polymorphisms or mutations you're looking for, the next key is to match patient samples against

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these variants and learn something that could guide treatment. Again, the technology to do this has been around for quite some time - since the 1960s, in fact, when gel electrophoresis was first used to identify DNA and proteins by using reagents and electromagnetic forces to "sift" samples through a gel and sort them by size or electrical charge.

But to be practical for large-scale screening, identification has to be rapid, cheap, automatable, and capable of dealing with large numbers of samples. As Dr. Toni Schuh, vice president of business development at Sequenom Inc. (San Diego, CA and Hamburg, Germany) puts it, companies must have a capacity for "industrial genomics." Another important ingredient for pharmacogenomic success is a truly awesome capacity for bioinformatics. After all, you don't just have to analyze and compare the DNA of hundreds to even tens of thousands of patients...you also have to correlate that data with patients' clinical information and drug use, which may be anything but uniform in its presentation. So add a lot of grunt data processing work to the shopping list, perhaps provided by an academic collaborator with access to plenty of cheap student labor.

The Bottom Line

So what you really need for success in pharmacogenomics is brute power in terms of sequencing and analysis; some approach to target validation; a lot of time and willpower for analyzing, recording, and standardizing patient information; and the ability to form research alliances that will provide access to those patients. Who can best provide all that?

While it seems like every company that ever made a DNA diagnostic is now talking about its role in pharmacogenomics, it is certainly makes sense that a variety of companies with cutting edge DNA sequencing and analysis technologies are coming forward to discuss how they might help the field advance. Some merely offer the tools; other sequencing powerhouses like Genset and Hyseq are pursuing the research themselves.

Given that there are many business risks inherent in pursuing pharmacogenomics as a business, at least in the near-term, companies that have other sources of revenue - ideally from other applications of their high-speed, high-capacity DNA analysis technologies - may make a better bet for the would-be pharmacogenomics investor.

On the other hand, the technical advantages of one analysis platform over another is by no means clear. Nor is it established that the leader in discovering gene variants will be the company most able to exploit that information.

Dedicated pharmacogenomic companies may fine-tune a system that proves superior to larger competitors with more raw horsepower. If the dedicated companies are to prevail, it will also likely be because only they give the necessary attention and expertise to the research. DNA analysis/genomics companies like Genset and Hyseq could discover that sorting through doctors' charts isn't something they want to do - or isn't something they can get their academic partners to do efficiently - and may be content to partner pharmacogenomic projects with specialists like Genaissance and Variagenics. But for now, the big guys are going to try it on their own.

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Even with these caveats, it is clear that high-octane analysis is vital for the advancement of pharmacogenomics. For that reason, BioVenture View took a look at some of the competing technologies that companies are hoping will become the backbone of the emerging field.

#### The Technologies

Gel electrophoresis has come a long way in terms of speed and automation since its debut in the 1960s, and some version of the technology is still used for the large-scale gene sequencing done by companies like Incyte, Genset, and HGS. Sales of electrophoresis gene sequencers and related products make a healthy business for companies like Perkin-Elmer Corp. (Norwalk, CT), Amersham Pharmacia Biotech Inc. (Uppsala, Sweden) and Molecular Dynamics Inc. (Sunnyvale, CA). But for pharmacogenomics, the issue isn't linear gene sequencing, it is the identification of single base-pair polymorphisms that might identify a patient as a candidate for a particular therapy. Gel electrophoresis can be - and is being - used for this purpose, too, but some companies are claiming to have technologies that will offer greater speed, accuracy, and flexibility.

Hybridization is the process by which a single strand of DNA matches up and binds with complementary base pairs. By making a series of "probes" - short strands of nucleotides in a known order - and seeing how completely a sample binds with them, researchers can perform highly accurate gene identification.

Traditionally, using a DNA probe array to identify gene mutations has required that you know exactly what sequence you're looking for. Affymetrix Inc. (Santa Clara, CA), for example, custom manufactures its chips with specific probe sequences already etched on the surface. If you've already identified all relevant polymorphisms of a gene from another sequencing approach, it makes sense to use a hybridization array as a diagnostic or quick screening tool.

Hybridization can also be used for large-scale gene sequencing. The process, sensibly enough called Sequencing By Hybridization (SBH), was developed by Hyseq. (The company claims exclusive rights to the technology, although they are currently involved in a patent infringement suit against Affymetrix regarding SBH.) Unlike traditional hybridization assays, SBH doesn't require the researcher to have any foreknowledge about the sequences they are studying.

Hyseq accomplishes this by using relatively short probes. A sequence of seven nucleotides in length only has 47, (about 16,000) possible combinations. Unlike the 24-mer probes used by Affymetrix, this makes for manageable number of combinations. The company actually creates a probe for every possible combination of seven nucleotides and uses it to screen unknown sequences.

(Another version of the technology can accommodate sequences up to 10 nucleotides in length by using two sets of 5-mer probes and looking for three-way binding.) Hybridization is being pursued by Hyseq as a core technology for all levels of sequencing, from large-scale discovery to pharmacogenomic diagnostics.

Another technology, called matrix-assisted laser desorption ionization/ time-of-flight (MALDI-TOF) mass spectrometry, also

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offers a highly accurate means of gene identification. For this application, a DNA sample is mixed with a light-absorbing molecule and hit with a laser pulse. A tiny cloud of DNA comes off the plate within an electrical field, and the mass of individual molecules is read by a mass spectrometer. The idea is that the molecules separate by size during flight, with the smaller ones have a shorter "time of flight" and reaching the detector first. MALDI-TOF mass spectrometry can also be used for proteomics - that is, it can do for proteins what it does for DNA. For this application, enzymes known to cleave the amino acids of protein samples at specific points are used to "digest" them.

Peptide-length protein probes are put in an array and run through the MALDI-TOF mass spec process, with resulting masses of the peptide sequences reassembled to uniquely identify the protein. Several companies that have pioneered the use of MALDI-TOF mass spectrometry have concentrated on protein applications and haven't discussed using their technology for pharmacogenomics.

For example, CipherGen Biosystems Ltd. (Palo Alto, CA and Surrey, U.K.) describes the applications of its SELDI (surface-enhanced laser desorption and ionization) ProteinChips as ligand-receptor assays, DNA-protein and protein-protein interaction studies, and immunoaffinity assays. PerSeptive Biosystems Inc., now part of Perkin-Elmer, also discusses its MALDI-TOF mass spectrometry technology in terms of proteomics.

But that could change. When you have technology that is useful in exploiting two of the hottest buzzwords around, it doesn't pay to limit yourself.

"We got into proteomics through DNA," says Dr. Noubar Afeyan, chairman, CEO, and founder of PerSeptive. He stresses that the use of the company's mass spec analysis for DNA "is a viable technology that we will commercialize. With Perkin-Elmer we now have a marketing channel to the DNA world. As PerSeptive, we mostly concentrated on the protein applications."

Other companies with MALDI-TOF mass spectrometry technology have concentrated on DNA applications from the start. GeneTrace Systems Inc. (Menlo Park, CA), originally a spin-out of SRI International Inc., has developed a variety of DNA analysis applications. In March 1996, Incyte licensed GeneTrace's laser mass spectrometry technology, took a minority stake in the company, and formed a partnership to develop specific DNA analysis applications.

Likewise, Sequenom's business plan calls for the pursuit of so-called industrial genomics, using rapid and highly accurate DNA analysis to do everything from gene mapping research to pharmacogenomic population analysis.

#### Genaissance and the Next Step

Other companies are using electrophoresis-based sequencing, with a variety of other strategies to speed the process. Variagenics, for example, uses a class of enzymes (called Resolvases) that cleave DNA wherever mismatched nucleotides are present - that is, wherever the DNA double helix doesn't hybridize. This helps the company quickly identify an area likely to represent a genetic problem, and according to the company accelerates a conventional gel-based sequencing process.

Visible Genetics Inc. (Toronto, Ontario, Canada) has specialized

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in making gene sequencing, software, and diagnostic tools that identify disease genetic subtypes, specifically in the area of HIV - working with technology licensed from Genaissance. The company's OpenGene System uses an "ultra-thin electrophoretic gel cassette" technology to identify disease variants. Essentially a multilane, high-throughput, automated gel electrophoresis system, the company believes its system is quicker and more cost-effective than probe hybridization technologies.

Genset has also used a gel-based approach as the core of its large-scale sequencing program. To identify genetic variants, the company uses its Functional Polymorphism Scanning (FPS) technique - an automated statistical analysis engine that compares sequences of interest from many unrelated individuals.

Genaissance's sequencing technology for mutation discovery, called Coupled Amplification and Screening (CAS), is also a gel electrophoresis-based system. But Ruano points out that the company has another technology, its pClasper functional screen for isogenes, that gives his company's approach its power.

"One of the problems in pharmacogenomics is that there is a lot of data being generated that has very little information content," said Ruano. "Polymorphisms don't mean anything - in our screening, we have found base pair changes every 50-100 bases. Some of these genes may have in the order of 20-25 polymorphisms.

"The question is, what does that mean?" Ruano continued. "There must be a way of quickly sorting out which of these genes are important." Enter pClasper, a shuttle vector that allows the company to easily take segments of DNA containing genes of interest and put them into yeast or bacterial cell lines, where they can be replicated in quantities suitable for high-throughput screening. The company was recently issued a U.S. Notice of Allowance on its pClasper patent.

"You can validate isogenes two ways," said Ruano. "One way is with real life studies" using expressed gene variants as drug targets and seeing how they behave. That's the goal behind the pClasper technology.

"The second way is through clinical outcomes," Ruano continued, "and I've got news for you - the two are connected." Genaissance hopes to partner with drug companies to do this kind of research during Phase II studies. He believes that's the best time to study genetic variants, since Phase III studies tend to have too many patients and come too far along the development pathway.

"Think about the size of a Phase II study - it has 200 to 300 patients. You need a very well-selected set of markers to be able to learn anything during a trial of that size", he said. "If you have 50,000 markers, how will you validate them in only 200 patients? How will you have the time to do that?"

One way or another, pharmacogenomic hypotheses will need real-life validation. "There are ways of getting some good information from retrospective studies," Ruano noted. "We are collecting some of that and analyzing correlations for existing drugs. But we are focused on developing drugs, so have to work with big pharma."

Who Has the Winner?

All three DNA analysis approaches - electrophoresis, hybridization, and mass spectrometry - have been developed into

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highly accurate, rapid systems. The ultimate advantage of one approach over the others will likely hinge on economics (in terms of both cost and speed) and the very margins of accuracy.

In the world of pharmacogenomics, accuracy takes on a whole new meaning. Identifying DNA polymorphisms with 99.99 percent accuracy means you'll make an average of 1 mistake every 10,000 base pairs. That's just not good enough, says Sequenom's Schuh. When you're looking for rare genetic anomalies among large populations, an error may be as common as the mutations or suspect alleles you're hoping to find.

Schuh thinks his company's MALDI-TOF approach is superior to hybridization, which uses an iterative approach to eliminate error. Hybridization requires that probes be "labeled" with fluorescent dyes that glow when a match is made. PerSeptive's Afeyan agrees that with mass spec technology, "it's big advantage that you don't have to fluorescently label your probes. Labeling is expensive and is a potential source of error." Ultimately, that could make a mass spec approach more cost-effective than hybridization.

On the other hand, throughput for mass spec analysis is currently limited. By definition, you can only measure the mass of one thing at a time - otherwise your results will be a meaningless sum of masses. Therefore, MALDI-TOF mass spec analysis is "fundamentally a serial process," says Afeyan. Companies like Sequenom are increasing the throughput capacity of mass spec DNA analysis by designing systems for parallel processing and multiplexing reactions, but they are still limited to measuring one mass at a time per mass spec tube.

In any case, Afeyan thinks predictions on the best DNA analysis technology are premature. "Chips and mass spectrometry and electrophoresis will have slight differences in accuracy, slight differences in throughput capability, and, for now, only slight differences in cost. It's very premature to say which of these approaches will be better. It's technology guesswork," he states. Perkin-Elmer is in an interesting position for this market, because they have access to every gene identification technology available. They continue to be a leading supplier of gel sequencers. They have an alliance with Hyseq to create HyChip hybridization arrays and software that could be used for pharmacogenomics. And they now own PerSeptive and its MALDI-TOF technology. It remains to be seen which technological horse - or horses - the company chooses to back for high-throughput pharmacogenomic DNA analysis.

#### Hyseq Charges Ahead

Their partner Hyseq, however, isn't waiting around. In February, the company announced a collaboration with the University of California-San Francisco (UCSF) to study genes that may play a role in cardiovascular disease. The project means sifting through a lot of sequence data to understand the complex interactions of genes, gene mutations, and polymorphisms that may play a role in disease.

UCSF researchers will collect DNA samples from 20,000 genetically diverse individuals and in many cases provide matching clinical histories, including the results of angiograms, and ultrasound and

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biochemical tests. Hyseq will create an annotated sequence database from the samples.

"We have right now 5,000 samples to process, and will collect an additional 15,000 samples," says Hyseq president and CEO Lewis Gruber. "We are in contact with the patients as well, and the study is intended to be expandable within the same area."

Gruber acknowledges that this large a study poses some daunting challenges in terms of processing and analyzing patient data. "The data normalization is to a large extent being done by UCSF people," he said. "We do have a large bioinformatics component to our company, with about 60 people working in that area."

"The first part of the project is polymorphism analysis," he continues, from which the company will assemble its database. "The pharmacogenomic portion is the next step - and again, we have access to these patients and can determine what drugs they are taking. We are looking to expand the polymorphism database into a pharmacogenomic information database on specific drugs."

Ultimately, the company intends to find pharmaceutical partners that will use the database for both existing and developmental drugs.

While Genaissance seeks to narrow the genetic variants it discovers through its screening technologies, Hyseq hopes to focus on the right genes by picking the right samples.

"The individual records in the database have been selected by our UCSF collaborators to provide important information - i.e. 80 year olds with no cardiovascular disease, 20 year olds with severe disease. And we'll have many people in each of these categories," explained Gruber. "That way you can select out the background."

Then you can target analysis to those genes that are useful for pharmaceuticals.

"Other companies have tended to take approaches that reduce the need for sequencing," he continued. "Our paradigm is the opposite: Because we have very fast sequencing, we can do what they can't because of cost and time. We can get more quickly at what people want, which is complete sequence analysis."

How does one small pharmacogenomic company feel about such a competitor? "That is a challenge for companies such as us," says Genaissance's Ruano. "But remember - for us, pharmacogenomics is not a me-too approach. This is our heart and soul; I've been thinking about this for years. Our technology has been developed specifically for this application."

DNA Analysis and pharmacogenomics - select approaches

Company	Technology
Affymetrix Inc. (Santa Clara, CA)	Custom photolithographic hybridization chip
ChemCore Inc. (Malvern, PA)	Diagnostic microchips
GeneTrace Systems Inc. (Menlo Park, CA)	MALDI-TOF DNA analysis
Genaissance Pharmaceuticals Inc. (New Haven, CT)	Gel-based sequencing and pClasper functional screening
Genset SA (Paris, France)	Gel-based sequencing and Functional Polymorphism Scanning

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Hyseq Inc. (Sunnyvale, CA)	"Generic" hybridization arrays and sequencing
Millennium Predictive Medicine Inc. (a division of Millennium Pharmaceuticals Inc.) (Cambridge, MA)	DNA, RNA, and protein analysis
Molecular Tool Inc. (a division of GeneScreen Inc.) (Baltimore, MD)	Hybridization chips
Nanogen Inc. (San Diego, CA)	Hybridization chips
PerSeptive BioSystems Inc. (a division of Perkin-Elmer Corp.) (Framingham, MA)	MALDI-TOF protein and DNA analysis
Rapigene Inc. (a division of Chiroscience plc) (Seattle, WA)	Hybridization arrays and mass spectrometry tags
Sequenom Inc. (Hamburg, Germany)	MALDI-TOF DNA analysis
Synteni Inc. (a division of Incyte Pharmaceuticals Inc.) (Palo Alto, CA)	Hybridization arrays and scanners for DNA analysis
Tm Bioscience Corp. (Toronto, Ontario, Canada)	Hybridization biochip
Variagenics Inc. (Cambridge, MA)	Gel-based sequencing and Enzymatic Mutation Detection to identify mismatched DNA
Visible Genetics Inc. (Toronto, Ontario, Canada)	Gel electrophoresis-based DNA analysis system

*Record - 61*

DIALOG(R) File 141:Readers Guide  
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04006026 H.W. WILSON RECORD NUMBER: BRGA99006026 (USE FORMAT 7 FOR FULLTEXT)

*The big sneeze.*

Elliott, Laura.

Washingtonian (Washingtonian) v. 33 no11 {i.e. 12} (Sept. '98) p. 76-81+

DOCUMENT TYPE: Feature Article

SPECIAL FEATURES: il ISSN: 0043-0897

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

RECORD TYPE: Abstract; Fulltext RECORD STATUS: Corrected or revised record

WORD COUNT: 9019

ABSTRACT: Forty million Americans suffer from allergies, but just one in ten sufferers seeks medical assistance. Allergy sufferers usually misdiagnose themselves as having colds and may purchase incorrect over-the-counter remedies that often worsen symptoms. Improperly treated, allergies can develop into more serious ear and sinus infections, bronchitis, nasal polyps, and even facial abnormalities. Advice on the symptoms associated with allergies, the treatment of allergies, and a list of the top allergists in the Washington, D.C., area are  
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N-[3(R)-[1-(5,6-Dihydro-4-hydroxy-2-oxo-6(S)-[2-phenylethyl]-6-propyl-2H-pyran-3-yl)-2,2-dimethylpropyl]phenyl]-1-methyl-1H-imidazole-4-sulfonamide;  
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 N-[3-{1(S)-(4-Hydroxy-5,6-dihydro-2-oxo-6,6-dipropyl-2H-pyran-3-yl)-propyl}phenyl]-5-cyanopyridine-2-sulfonamide;  
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 N-[3-{1(R)-(4-Hydroxy-5,6-dihydro-2-oxo-6(S)-phenethyl-6-propyl-2H-pyran-3-yl)propyl}phenyl]-5-cyanopyridine-2-sulfonamide;  
 N-[3-{1(S)-(4-Hydroxy-5,6-dihydro-2-oxo-6(R)-phenethyl-6-propyl-2H-pyran-3-yl)propyl}phenyl]-5-cyanopyridine-2-sulfonamide; and  
 N-[3-{1(S)-(4-Hydroxy-5,6-dihydro-2-oxo-6(S)-phenethyl-6-propyl-2H-pyran-3-yl)propyl}phenyl]-5-cyanopyridine-2-sulfonamide.

**Record - 202**

DIALOG(R) File 654:US Pat.Full.

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03241214

Utility

MINIATURIZED GENETIC ANALYSIS SYSTEMS AND METHODS

PATENT NO.: 6,168,948

ISSUED: January 02, 2001 (20010102)

INVENTOR(s): Anderson, Rolfe C., Saratoga, CA (California), US (United States of America)

Lipshutz, Robert J., Palo Alto, CA (California), US (United States of America)

Rava, Richard P., Redwood City, CA (California), US (United States of America)

Fodor, Stephen P. A., Palo Alto, CA (California), US (United States of America)

ASSIGNEE(s): Affymetrix, Inc., (A U.S. Company or Corporation), Santa Clara, CA (California), US (United States of America)

[Assignee Code(s): 39771]

APPL. NO.: 9-5,985

FILED: January 12, 1998 (19980112)

**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No. 60-043,490, filed Apr. 10, 1997. This application is a continuation-in-part of U.S. application Ser. No. 08-992,025, filed Dec. 17, 1997, now abandoned; and is a (cont. next page)

continuation-in-part of U.S. application Ser. No. 08-589,027, filed Jan. 19, 1996, now U.S. Pat. No. 5,856,174; and is a continuation-in-part of U.S. application Ser. No. 08-671,928, filed Jun. 27, 1996, now U.S. Pat. No. 5,922,591, which claims the benefit of U.S. Provisional Application No. 60-000,703, filed Jun. 29, 1995, and U.S. Provisional Application No. 60-000,859, filed Jul. 5, 1995. Each of these applications is incorporated herein by reference in its entirety for all purposes.

## GOVERNMENT RIGHTS

Portions of the present invention were made with U.S. Government support under ATP Grant No. 7ONANB5H1031. The government may have certain rights in this invention.

U.S. CLASS: 435-287.2 cross ref: 435-6; 435-287.9; 435-288.6

INTL CLASS: [7] C12M 1-34

FIELD OF SEARCH: 435-6; 435-7.1; 435-7.92; 435-287.1; 435-287.2; 435-287.9;  
435-288.6; 436-518; 436-523; 436-527; 436-528; 436-530; 436-89; 436-90  
; 436-94; 422-68.1; 422-100; 422-101; 422-102; 536-25.4; 536-75.41;  
210-656; 210-198.2

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PRIMARY EXAMINER: Beisner, William H.

ATTORNEY, AGENT, OR FIRM: Townsend and Townsend and Crew LLP

CLAIMS: 6

EXEMPLARY CLAIM: 1,3,4

DRAWING PAGES: 62

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ART UNIT: 174

#### ABSTRACT

The present invention provides a miniaturized integrated nucleic acid diagnostic device and system which includes a nucleic acid extraction zone including nucleic acid binding sites.

What is claimed is:

1. A nucleic acid extraction device, comprising:  
a body having at least one chamber with at least one inlet channel;  
a porous flow-through deformable plug disposed within the chamber, the deformable plug having nucleic acid binding properties; and  
a flexible diaphragm for compressing said plug thereby removing trapped liquids.

2. The nucleic acid extraction device of claim 1, wherein  
the flexible diaphragm is disposed between a pneumatic port and the deformable plug, the device further comprising a pressure system for displacing the flexible diaphragm to draw a sample through the inlet channel into the chamber.

3. A nucleic acid extraction device, comprising:  
a body having at least one chamber with at least one inlet channel, wherein the chamber comprises a textured interior wall surface having nucleic acid binding properties;

(cont. next page)

a porous flow-through plug disposed within the chamber, the plug having nucleic acid binding properties, and

a piezoelectric crystal adapted to acoustically agitate a nucleic acid sample, and wherein the piezoelectric crystal is mounted to the chamber opposite the textured interior wall surface of the chamber.

4. A biological sample refinement device, comprising:

a body having at least one microchamber with at least one inlet channel;

a structure disposed within the microchamber, the structure having binding sites thereon; and

a fluid distribution system for delivering a biological sample into the microchamber such that at least a portion of the sample contacts the binding sites, the fluid distribution system being adapted to deliver an adjustable volume of metered elution buffer into the microchamber.

5. The device of claim 4, wherein the structure comprises a substantially planar wall with a plurality of beads attached thereto.

6. The device of claim 4, wherein,

the binding sites comprise agents selected from the group consisting of acids, bases, silanes, polylysine, tethered antibodies, nucleic acids and Poly-T DNA.

*Record - 203*

DIALOG(R) File 654:US Pat.Full.

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Utility  
FABF

PATENT NO.: 6,168,797

ISSUED: January 02, 2001 (20010102)

INVENTOR(s): Biswas, Sanjoy, Paoli, PA (Pennsylvania), US (United States of America)

Burnham, Martin K R, Barto, PA (Pennsylvania), US (United States of America)

Fedon, Jason C, Strafford, PA (Pennsylvania), US (United States of America)

Holmes, David J, West Chester, PA (Pennsylvania), US (United States of America)

Ingraham, Karen A, Auburn, PA (Pennsylvania), US (United States of America)

Kallender, Howard, Wayne, PA (Pennsylvania), US (United States of America)

Lonsdale, John T, Exton, PA (Pennsylvania), US (United States of America)

Mazzulla, Marie J, Collegeville, PA (Pennsylvania), US (United States of America)

O'Dwyer, Karen M, Phoenixville, PA (Pennsylvania), US (United States of America)

Palmer, Leslie M, Audubon, PA (Pennsylvania), US (United States of America)

(cont. next page)

Responses to toxins show how adaptive changes can be based on major genes with polygenic modifiers. Analyses of continuous variation in ecologically relevant traits indicate low levels of heritable variation in some natural situations and highlight the importance of genetic interactions. The role of major and minor genes in adaptive responses in quantitative traits is unclear. Some evidence suggests that genetic trade-offs exist among environments and life history traits.

## DESCRIPTORS:

Population genetics; Quantitative genetics; Genetics and environment

## Record - 59

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*IFT EXPO - same game, different name*

Food Manufacture , p 46

September, 1999

LANGUAGE: English RECORD TYPE: Fulltext DOC. TYPE: Journal

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2869 .(Industrial Organic Chemicals, NEC) - Company acquisition

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2099 .(Food Preparations, NEC) - Product information

2087 .(Flavoring Extracts and Syrups, NEC) - Product information

2046 .(Wet Corn Milling) - Product information

2869 .(Industrial Organic Chemicals, NEC) - Product information

2023 .(Dry, Condensed, Evaporated Products) - Product information

2077 .(Animal and Marine Fats and Oils) - Product information

2086 .(Bottled and Canned Soft Drinks) - Product information

2066 .(Chocolate and Cocoa Products) - Product information

3556 .(Food Products Machinery) - Product information

204 .(Grain Mill Products) - Product information

COMPANY NAMES: Danisco-Cultor; McNeil Speciality Products; Cerestar; DSM Food Specialities; Monsanto; Lucas Meyer Group; SKW Trostberg; FMC; Pronova Biopolymer; Pronova; Avonmore Waterford Ingredients; Roche Vitamins; Watson; Folexco; National Starch and Chemical; Armfield; Chaucer Food Group; Kerry Inc

COUNTRY: Norway;

REGION: West Europe; USA

## Record - 60

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00577672

*Pharmacogenomic Strategies: Biotech's New David and Goliath Challenge*

Bioventure-View 1304 pl, April 01, 1998 (19980401)

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(cont. next page)



You've heard the buzzword. Lots of companies are talking about their move into pharmacogenomics, the analysis of genetic differences among patients that could mark them as better or worse candidates for a particular drug ... or lead to highly targeted, even "personalized" drugs.

If you can efficiently diagnose a patient in terms of DNA mutations, alleles, or polymorphisms pertaining to a specific disease, you'll potentially have vital information for improving their response to treatment.

Pharmacogenomics is an idea that has just been waiting for the technology to make it possible. The basic ingredients are nothing new. DNA-based diagnostics, for instance, have been sold for almost 20 years. Likewise, the desire to identify subgroups of a population most likely to benefit from drugs is an impulse with some history behind it.

For commercial pharmacogenomics to be practical, researchers need a method for high capacity, cost-effective, rapid DNA identification and a large amount of existing sequence data so patient genes can be compared to the "norm."

Both of these key areas have only recently reached critical mass. The initiation of the Human Genome Project in 1992 started the race to sequence the complete human genome. Many companies, including Human Genome Sciences Inc. (Rockville, MD), Incyte Pharmaceuticals Inc. (Palo Alto, CA), Millennium Pharmaceuticals Inc. (Cambridge, MA), Genset SA (Paris, France), AxyS Pharmaceuticals Inc. (South San Francisco, CA), and Hyseq Inc. (Sunnyvale, CA), have built up large databases of linear sequence information, and huge amounts of publicly available sequence data have also been amassed.

That's just the beginning, however. Having a sequence of the entire human genome - or the entire genomes of several individuals - tells you little about the genetic mutations and polymorphisms that may be implicated in a specific disease. That work still needs to be done, and has led to the birth of several "pure play" pharmacogenomic companies like Genaissance Pharmaceuticals Inc. (New Haven, CT) and Variagenics Inc. (Cambridge, MA), as well as a major initiative Hyseq and by Genset (in collaboration with Abbott Laboratories).

#### Lots of Work To Do

Behind these efforts are advances in technology that will allow small portions of the genome involved in a disease to be sequenced over and over again in thousands of people and matched against individuals' clinical information on symptoms, treatments, and outcomes.

Discovering mutants, alleles, polymorphisms - what Genaissance calls "isogenes" - is just the first step. "The key thing is that you don't just have to discover things, you have to validate them," says Dr. Gualberto Ruano, founder and CEO of Genaissance. Companies like his that are dedicated to the creation of personalized medicines are hoping they have the key technology that can make the leap from numerous DNA anomalies to a few key disease-related gene variants.

Once you've learned which polymorphisms or mutations you're looking for, the next key is to match patient samples against

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these variants and learn something that could guide treatment. Again, the technology to do this has been around for quite some time - since the 1960s, in fact, when gel electrophoresis was first used to identify DNA and proteins by using reagents and electromagnetic forces to "sift" samples through a gel and sort them by size or electrical charge.

But to be practical for large-scale screening, identification has to be rapid, cheap, automatable, and capable of dealing with large numbers of samples. As Dr. Toni Schuh, vice president of business development at Sequenom Inc. (San Diego, CA and Hamburg, Germany) puts it, companies must have a capacity for "industrial genomics." Another important ingredient for pharmacogenomic success is a truly awesome capacity for bioinformatics. After all, you don't just have to analyze and compare the DNA of hundreds to even tens of thousands of patients...you also have to correlate that data with patients' clinical information and drug use, which may be anything but uniform in its presentation. So add a lot of grunt data processing work to the shopping list, perhaps provided by an academic collaborator with access to plenty of cheap student labor.

The Bottom Line

So what you really need for success in pharmacogenomics is brute power in terms of sequencing and analysis; some approach to target validation; a lot of time and willpower for analyzing, recording, and standardizing patient information; and the ability to form research alliances that will provide access to those patients. Who can best provide all that?

While it seems like every company that ever made a DNA diagnostic is now talking about its role in pharmacogenomics, it is certainly makes sense that a variety of companies with cutting edge DNA sequencing and analysis technologies are coming forward to discuss how they might help the field advance. Some merely offer the tools; other sequencing powerhouses like Genset and Hyseq are pursuing the research themselves.

Given that there are many business risks inherent in pursuing pharmacogenomics as a business, at least in the near-term, companies that have other sources of revenue - ideally from other applications of their high-speed, high-capacity DNA analysis technologies - may make a better bet for the would-be pharmacogenomics investor.

On the other hand, the technical advantages of one analysis platform over another is by no means clear. Nor is it established that the leader in discovering gene variants will be the company most able to exploit that information.

Dedicated pharmacogenomic companies may fine-tune a system that proves superior to larger competitors with more raw horsepower. If the dedicated companies are to prevail, it will also likely be because only they give the necessary attention and expertise to the research. DNA analysis/genomics companies like Genset and Hyseq could discover that sorting through doctors' charts isn't something they want to do - or isn't something they can get their academic partners to do efficiently - and may be content to partner pharmacogenomic projects with specialists like Genaissance and Variagenics. But for now, the big guys are going to try it on their own.

(cont. next page)

Even with these caveats, it is clear that high-octane analysis is vital for the advancement of pharmacogenomics. For that reason, BioVenture View took a look at some of the competing technologies that companies are hoping will become the backbone of the emerging field.

#### The Technologies

Gel electrophoresis has come a long way in terms of speed and automation since its debut in the 1960s, and some version of the technology is still used for the large-scale gene sequencing done by companies like Incyte, Genset, and HGS. Sales of electrophoresis gene sequencers and related products make a healthy business for companies like Perkin-Elmer Corp.

(Norwalk, CT), Amersham Pharmacia Biotech Inc. (Uppsala, Sweden) and Molecular Dynamics Inc. (Sunnyvale, CA). But for

pharmacogenomics, the issue isn't linear gene sequencing, it is the identification of single base-pair polymorphisms that might identify a patient as a candidate for a particular therapy. Gel electrophoresis can be - and is being - used for this purpose, too, but some companies are claiming to have technologies that will offer greater speed, accuracy, and flexibility.

Hybridization is the process by which a single strand of DNA matches up and binds with complementary base pairs. By making a series of "probes" - short strands of nucleotides in a known order - and seeing how completely a sample binds with them, researchers can perform highly accurate gene identification.

Traditionally, using a DNA probe array to identify gene mutations has required that you know exactly what sequence you're looking for. Affymetrix Inc. (Santa Clara, CA), for example, custom manufactures its chips with specific probe sequences already etched on the surface. If you've already identified all relevant polymorphisms of a gene from another sequencing approach, it makes sense to use a hybridization array as a diagnostic or quick screening tool.

Hybridization can also be used for large-scale gene sequencing. The process, sensibly enough called Sequencing By Hybridization (SBH), was developed by Hyseq. (The company claims exclusive rights to the technology, although they are currently involved in a patent infringement suit against Affymetrix regarding SBH.) Unlike traditional hybridization assays, SBH doesn't require the researcher to have any foreknowledge about the sequences they are studying.

Hyseq accomplishes this by using relatively short probes. A sequence of seven nucleotides in length only has 47, (about 16,000) possible combinations. Unlike the 24-mer probes used by Affymetrix, this makes for manageable number of combinations. The company actually creates a probe for every possible combination of seven nucleotides and uses it to screen unknown sequences.

(Another version of the technology can accommodate sequences up to 10 nucleotides in length by using two sets of 5-mer probes and looking for three-way binding.) Hybridization is being pursued by Hyseq as a core technology for all levels of sequencing, from large-scale discovery to pharmacogenomic diagnostics.

Another technology, called matrix-assisted laser desorption ionization/ time-of-flight (MALDI-TOF) mass spectrometry, also

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offers a highly accurate means of gene identification. For this application, a DNA sample is mixed with a light-absorbing molecule and hit with a laser pulse. A tiny cloud of DNA comes off the plate within an electrical field, and the mass of individual molecules is read by a mass spectrometer. The idea is that the molecules separate by size during flight, with the smaller ones have a shorter "time of flight" and reaching the detector first. MALDI-TOF mass spectrometry can also be used for proteomics - that is, it can do for proteins what it does for DNA. For this application, enzymes known to cleave the amino acids of protein samples at specific points are used to "digest" them. Peptide-length protein probes are put in an array and run through the MALDI-TOF mass spec process, with resulting masses of the peptide sequences reassembled to uniquely identify the protein. Several companies that have pioneered the use of MALDI-TOF mass spectrometry have concentrated on protein applications and haven't discussed using their technology for pharmacogenomics. For example, CipherGen Biosystems Ltd. (Palo Alto, CA and Surrey, U.K.) describes the applications of its SELDI (surface-enhanced laser desorption and ionization) ProteinChips as ligand-receptor assays, DNA-protein and protein-protein interaction studies, and immunoaffinity assays. PerSeptive Biosystems Inc., now part of Perkin-Elmer, also discusses its MALDI-TOF mass spectrometry technology in terms of proteomics. But that could change. When you have technology that is useful in exploiting two of the hottest buzzwords around, it doesn't pay to limit yourself. "We got into proteomics through DNA," says Dr. Noubar Afeyan, chairman, CEO, and founder of PerSeptive. He stresses that the use of the company's mass spec analysis for DNA "is a viable technology that we will commercialize. With Perkin-Elmer we now have a marketing channel to the DNA world. As PerSeptive, we mostly concentrated on the protein applications." Other companies with MALDI-TOF mass spectrometry technology have concentrated on DNA applications from the start. GeneTrace Systems Inc. (Menlo Park, CA), originally a spin-out of SRI International Inc., has developed a variety of DNA analysis applications. In March 1996, Incyte licensed GeneTrace's laser mass spectrometry technology, took a minority stake in the company, and formed a partnership to develop specific DNA analysis applications. Likewise, Sequenom's business plan calls for the pursuit of so-called industrial genomics, using rapid and highly accurate DNA analysis to do everything from gene mapping research to pharmacogenomic population analysis. Genaissance and the Next Step Other companies are using electrophoresis-based sequencing, with a variety of other strategies to speed the process. Variagenics, for example, uses a class of enzymes (called Resolvases) that cleave DNA wherever mismatched nucleotides are present - that is, wherever the DNA double helix doesn't hybridize. This helps the company quickly identify an area likely to represent a genetic problem, and according to the company accelerates a conventional gel-based sequencing process. Visible Genetics Inc. (Toronto, Ontario, Canada) has specialized

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in making gene sequencing, software, and diagnostic tools that identify disease genetic subtypes, specifically in the area of HIV - working with technology licensed from Genaissance. The company's OpenGene System uses an "ultra-thin electrophoretic gel cassette" technology to identify disease variants. Essentially a multilane, high-throughput, automated gel electrophoresis system, the company believes its system is quicker and more cost-effective than probe hybridization technologies.

Genset has also used a gel-based approach as the core of its large-scale sequencing program. To identify genetic variants, the company uses its Functional Polymorphism Scanning (FPS) technique - an automated statistical analysis engine that compares sequences of interest from many unrelated individuals.

Genaissance's sequencing technology for mutation discovery, called Coupled Amplification and Screening (CAS), is also a gel electrophoresis-based system. But Ruano points out that the company has another technology, its pClasper functional screen for isogenes, that gives his company's approach its power.

"One of the problems in pharmacogenomics is that there is a lot of data being generated that has very little information content," said Ruano. "Polymorphisms don't mean anything - in our screening, we have found base pair changes every 50-100 bases. Some of these genes may have in the order of 20-25 polymorphisms.

"The question is, what does that mean?" Ruano continued. "There must be a way of quickly sorting out which of these genes are important." Enter pClasper, a shuttle vector that allows the company to easily take segments of DNA containing genes of interest and put them into yeast or bacterial cell lines, where they can be replicated in quantities suitable for high-throughput screening. The company was recently issued a U.S. Notice of Allowance on its pClasper patent.

"You can validate isogenes two ways," said Ruano. "One way is with real life studies" using expressed gene variants as drug targets and seeing how they behave. That's the goal behind the pClasper technology.

"The second way is through clinical outcomes," Ruano continued, "and I've got news for you - the two are connected." Genaissance hopes to partner with drug companies to do this kind of research during Phase II studies. He believes that's the best time to study genetic variants, since Phase III studies tend to have too many patients and come too far along the development pathway.

"Think about the size of a Phase II study - it has 200 to 300 patients. You need a very well-selected set of markers to be able to learn anything during a trial of that size", he said. "If you have 50,000 markers, how will you validate them in only 200 patients? How will you have the time to do that?"

One way or another, pharmacogenomic hypotheses will need real-life validation. "There are ways of getting some good information from retrospective studies," Ruano noted. "We are collecting some of that and analyzing correlations for existing drugs. But we are focused on developing drugs, so have to work with big pharma."

Who Has the Winner?

All three DNA analysis approaches - electrophoresis, hybridization, and mass spectrometry - have been developed into

(cont. next page)

highly accurate, rapid systems. The ultimate advantage of one approach over the others will likely hinge on economics (in terms of both cost and speed) and the very margins of accuracy. In the world of pharmacogenomics, accuracy takes on a whole new meaning. Identifying DNA polymorphisms with 99.99 percent accuracy means you'll make an average of .1 mistake every 10,000 base pairs. That's just not good enough, says Sequenom's Schuh. When you're looking for rare genetic anomalies among large populations, an error may be as common as the mutations or suspect alleles you're hoping to find.

Schuh thinks his company's MALDI-TOF approach is superior to hybridization, which uses an iterative approach to eliminate error. Hybridization requires that probes be "labeled" with fluorescent dyes that glow when a match is made. PerSeptive's Afeyan agrees that with mass spec technology, "it's big advantage that you don't have to fluorescently label your probes. Labeling is expensive and is a potential source of error." Ultimately, that could make a mass spec approach more cost-effective than hybridization.

On the other hand, throughput for mass spec analysis is currently limited. By definition, you can only measure the mass of one thing at a time - otherwise your results will be a meaningless sum of masses. Therefore, MALDI-TOF mass spec analysis is "fundamentally a serial process," says Afeyan. Companies like Sequenom are increasing the throughput capacity of mass spec DNA analysis by designing systems for parallel processing and multiplexing reactions, but they are still limited to measuring one mass at a time per mass spec tube.

In any case, Afeyan thinks predictions on the best DNA analysis technology are premature. "Chips and mass spectrometry and electrophoresis will have slight differences in accuracy, slight differences in throughput capability, and, for now, only slight differences in cost. It's very premature to say which of these approaches will be better. It's technology guesswork," he states. Perkin-Elmer is in an interesting position for this market, because they have access to every gene identification technology available. They continue to be a leading supplier of gel sequencers. They have an alliance with Hyseq to create HyChip hybridization arrays and software that could be used for pharmacogenomics. And they now own PerSeptive and its MALDI-TOF technology. It remains to be seen which technological horse - or horses - the company chooses to back for high-throughput pharmacogenomic DNA analysis.

#### Hyseq Charges Ahead

Their partner Hyseq, however, isn't waiting around. In February, the company announced a collaboration with the University of California-San Francisco (UCSF) to study genes that may play a role in cardiovascular disease. The project means sifting through a lot of sequence data to understand the complex interactions of genes, gene mutations, and polymorphisms that may play a role in disease.

UCSF researchers will collect DNA samples from 20,000 genetically diverse individuals and in many cases provide matching clinical histories, including the results of angiograms, and ultrasound and

(cont. next page)

biochemical tests. Hyseq will create an annotated sequence database from the samples.

"We have right now 5,000 samples to process, and will collect an additional 15,000 samples," says Hyseq president and CEO Lewis Gruber. "We are in contact with the patients as well, and the study is intended to be expandable within the same area." Gruber acknowledges that this large a study poses some daunting challenges in terms of processing and analyzing patient data. "The data normalization is to a large extent being done by UCSF people," he said. "We do have a large bioinformatics component to our company, with about 60 people working in that area." "The first part of the project is polymorphism analysis," he continues, from which the company will assemble its database. "The pharmacogenomic portion is the next step - and again, we have access to these patients and can determine what drugs they are taking. We are looking to expand the polymorphism database into a pharmacogenomic information database on specific drugs." Ultimately, the company intends to find pharmaceutical partners that will use the database for both existing and developmental drugs.

While Genaissance seeks to narrow the genetic variants it discovers through its screening technologies, Hyseq hopes to focus on the right genes by picking the right samples.

"The individual records in the database have been selected by our UCSF collaborators to provide important information - i.e. 80 year olds with no cardiovascular disease, 20 year olds with severe disease. And we'll have many people in each of these categories," explained Gruber. "That way you can select out the background. Then you can target analysis to those genes that are useful for pharmaceuticals."

"Other companies have tended to take approaches that reduce the need for sequencing," he continued. "Our paradigm is the opposite: Because we have very fast sequencing, we can do what they can't because of cost and time. We can get more quickly at what people want, which is complete sequence analysis."

How does one small pharmacogenomic company feel about such a competitor? "That is a challenge for companies such as us," says Genaissance's Ruano. "But remember - for us, pharmacogenomics is not a me-too approach. This is our heart and soul; I've been thinking about this for years. Our technology has been developed specifically for this application."

DNA Analysis and pharmacogenomics -	select approaches
Company	Technology
Affymetrix Inc.	Custom photolithographic
(Santa Clara, CA)	hybridization chip
ChemCore Inc.	Diagnostic microchips
(Malvern, PA)	
GeneTrace Systems Inc.	MALDI-TOF DNA analysis
(Menlo Park, CA)	
Genaissance Pharmaceuticals Inc.	Gel-based sequencing and
(New Haven, CT)	pClasper functional screening
Genset SA	Gel-based sequencing and
(Paris, France)	Functional Polymorphism
	Scanning

(cont. next page)

DIALOG

Hyseq Inc. (Sunnyvale, CA)	"Generic" hybridization arrays and sequencing
Millennium Predictive Medicine Inc. (a division of Millennium Pharmaceuticals Inc.) (Cambridge, MA)	DNA, RNA, and protein analysis
Molecular Tool Inc. (a division of GeneScreen Inc.) (Baltimore, MD)	Hybridization chips
Nanogen Inc. (San Diego, CA)	Hybridization chips
PerSeptive BioSystems Inc. (a division of Perkin-Elmer Corp.) (Framingham, MA)	MALDI-TOF protein and DNA analysis
Rapigene Inc. (a division of Chiroscience plc) (Seattle, WA)	Hybridization arrays and mass spectrometry tags
Sequenom Inc. (Hamburg, Germany)	MALDI-TOF DNA analysis
Synteni Inc. (a division of Incyte Pharmaceuticals Inc.) (Palo Alto, CA)	Hybridization arrays and scanners for DNA analysis
Tm Bioscience Corp. (Toronto, Ontario, Canada)	Hybridization biochip
Variagenics Inc. (Cambridge, MA)	Gel-based sequencing and Enzymatic Mutation Detection to identify mismatched DNA
Visible Genetics Inc. (Toronto, Ontario, Canada)	Gel electrophoresis-based DNA analysis system

## Record - 61

DIALOG(R) File 141:Readers Guide  
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04006026 H.W. WILSON RECORD NUMBER: BRGA99006026 (USE FORMAT 7 FOR FULLTEXT)  
*The big sneeze.*

Elliott, Laura.

Washingtonian (Washingtonian) v. 33 no11 {i.e. 12} (Sept. '98) p. 76-81+

DOCUMENT TYPE: Feature Article

SPECIAL FEATURES: ii ISSN: 0043-0897

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

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WORD COUNT: 9019

ABSTRACT: Forty million Americans suffer from allergies, but just one in ten sufferers seeks medical assistance. Allergy sufferers usually misdiagnose themselves as having colds and may purchase incorrect over-the-counter remedies that often worsen symptoms. Improperly treated, allergies can develop into more serious ear and sinus infections, bronchitis, nasal polyps, and even facial abnormalities. Advice on the symptoms associated with allergies, the treatment of allergies, and a list of the top allergists in the Washington, D.C., area are  
(cont. next page)



than 0.01 V/K moles of binding agent.

13. A method as claimed in claim 11, wherein said binding agents used have affinity constants for said analytes of from  $10^8$  to  $10^{13}$  liters per mole.

14. A method as claimed in claim 11, wherein said binding agents used have affinity constants for said analytes of the order of  $10^{10}$  to  $10^{11}$  liters per mole.

15. A method as claimed in claim 11, wherein the volume of said liquid sample is not more than 0.1 liter.

16. A method as claimed in claim 11, wherein the volume of said liquid sample is 400 to 1000 microliters.

17. A method as claimed in claim 9, wherein said binding agents loaded onto said support means are antibodies for the analytes whose concentrations are to be determined.

1/5/4 (Item 2 from file: 654)

DIALOG(R) File 654:US Pat.Full.

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02492517

Utility

DEVICES AND KITS FOR IMMUNOLOGICAL ANALYSIS

PATENT NO.: 5,486,452

ISSUED: January 23, 1996 (19960123)

INVENTOR(s): Gordon, Julian, Arlesheim, CH (Switzerland)

Hawkes, Richard, Allschwil, CH (Switzerland)

Niday, Evelyn, Arlington Heights, IL (Illinois), US (United States of America)

Towbin, Harry, Allschwil, CH (Switzerland)

ASSIGNEE(s): Ciba-Geigy Corporation, (A U.S. Company or Corporation),

Ardsley, NY (New York), US (United States of America)

[Assignee Code(s): 2]

EXTRA INFO: Assignment transaction [Reassigned], recorded August 22,

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Assignor(s): CIBA-GEIGY CORPORATION -- signed: 06/12/1997

Recorded: August 22, 2000 (20000822)

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Brief: CHANGE OF NAME

Rep.: NOVARTIS CORPORATION THOMAS HOXIE 564 MORRIS

AVENUE SUMMIT, NJ 07901-1027

APPL. NO.: 7-38,470

FILED: April 10, 1987 (19870410)

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This application is a continuation of application Ser. No. 06-912,144, filed Sep. 24, 1986 and now abandoned, which is a continuation of application Ser. No. 06-673,211; filed Nov. 19, 1984 and now abandoned, which is a continuation of application Ser. No. 06-345,440, filed Feb. 3, 1982 and now abandoned.

U.S. CLASS: 435-5 cross ref: 435-7.1; 435-7.2; 435-7.21; 435-7.22;

435-7.23; 435-7.31; 435-7.32; 435-7.7; 435-7.72; 435-7.9;

435-7.91; 435-7.92; 435-7.94; 435-7.95; 435-970; 435-975;

436-506; 436-507; 436-509; 436-518; 436-530; 436-807; 436-821

INTL CLASS: [6] G01N 33-548; G01N 33-564; G01N 33-569

FIELD OF SEARCH: 436-506; 436-507; 436-509; 436-518; 436-530; 436-807;

436-821; 436-810; 435-7.1.23; 435-7.31; 435-7.32; 435-7.7;  
435-7.72; 435-7.9.95; 435-970; 435-975

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PRIMARY EXAMINER: Saunders, David  
 ATTORNEY, AGENT, OR FIRM: Fishman, Irving M.; Kaiser, Karen G.  
 CLAIMS: 47  
 EXEMPLARY CLAIM: 1  
 DRAWING PAGES: 1  
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 ART UNIT: 182

#### ABSTRACT

New devices and kits for solid-phase immuno-assays comprising a solid porous support, preferably in the form of a sheet, where antigens or immuno-globulins or both of them are bound by direct application in any suitable geometry, e.g. as an assay of dots or lines. Such porous supports are suitable for effecting an unlimited number of antibody-antigen reactions simultaneously and in one operation.  
 What is claimed:

1. An immunological analysis device consisting of a porous sheet of nitrocellulose containing an array of preselected geometry of delimited absorption areas of at least one compound capable of specifically binding an antigen (antigen-reactive compound) which adheres tightly and does not spread out on the surface of said porous sheet, said array of preselected geometry being obtained in said porous sheet by applying liquid aliquots of said antigen-reactive compound to said porous sheet mechanically via direct contact, wherein residual absorption sited in said porous sheet are unsaturated or saturated by non-specific protein.

2. The device of claim 1 wherein said antigen-reactive compound is an immunoglobulin of human or animal origin or a binding fragment thereof for the detection and quantitation of rheumatoid factor.

3. The device of claim 1 wherein said antigen-reactive compound is a complement protein for the detection and quantitation of a circulating immune complex which circulating immune complex comprises an antigen-antibody complex.

4. The device of claim 1 wherein said porous sheet is from about 0.01 to

about 0.5 mm thick.

5. The device of claim 4 wherein said porous sheet is about 0.1 mm thick.
6. The device of claim 1 wherein said nitrocellulose has about 3 nitric acid groups per 6 carbon atoms.
7. The device of claim 1 wherein said porous sheet has an average pore size of about 0.45  $\mu$ m.
8. The device of claim 1 wherein said array is an array of dots.
9. The device of claim 1 wherein said array is an array of microdots having diameters less than 2 mm.
10. The device of claim 1 wherein said array is an array of lines of width of 2 mm or less.
11. The device of claim 1 wherein said antigen-reactive compound is applied to said support by a capillary tube, pipette, syringe, or spray.
12. The device of claim 1 wherein said antigen-reactive compound is applied to said support in volumes of less than 1  $\mu$ l.
13. The device of claim 1 wherein said residual adsorption area is saturated with a serum containing substance, wherein said substance is undiluted total serum or total serum diluted with sodium chloride.
14. The device of claim 1 wherein said antigen-reactive compound is reactive to an antigen, wherein said antigen is a constituent of human biopsy material, mammalian tissue, mammalian cells, body fluids, fungi, protozoa, metazoan parasites, bacteria, mycoplasma, or viruses.
15. A kit comprising the device of claim 1 and reagents of an indicator system in pre-aliquoted or dessicated form to allow detection of an antigen-antigen reactive compound complex.
16. The kit of claim 15 wherein said indicator is selected from the group consisting of
  - A) antibodies capable of forming antigen-antibody complexes, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate,
  - B) complement protein capable of binding to antigen-antibody complexes, wherein the complement protein is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving color reaction with a suitable substrate, and
  - C) complement protein and a further specific anti-complement antibody, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate.
17. The kit of claim 16, wherein said antigen-reactive compound is complement protein for the detection and quantitation of circulating immune complex which circulating immune complex comprises an antigen-antibody complex and wherein the indicator is a group A) antibody radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate.
18. The kit of claim 15, wherein said antigen-reactive compound is an immunoglobulin of human or animal origin or a binding fragment thereof for the detection and quantitation of rheumatoid factor.
19. The kit of claim 15, comprising a multi-cavity plastic tray, lyophilized mixtures of indicator antibody, salts, buffers, carrier serum or protein, and pre-determined amounts of indicator enzyme chromogenic substrate, salts, buffers, and ampoules containing pre-measured volumes of liquid substrates, all in suitable packaging.

20. The kit of claim 15 wherein said reagents of said indicator system comprise a substrate, a cofactor or prosthetic group for an indicator enzyme, or a coupled series of enzymes.

21. The kit of claim 15, wherein the reagents of said indicator system comprise a covalent adduct between an antigen and a signalling molecule.

22. A method of immunological analysis comprising

(a) incubating the device of claim 1 wherein residual absorption sites in said porous sheet are saturated by non-specific protein, with a sample containing an immunoglobulin or other antigen to be detected;

(b) incubating said device with a solution of an indicator selected from the group consisting of

A) antibodies capable of forming antigen-antibody complexes, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate,

B) complement protein capable of binding to antigen-antibody complexes, wherein the complement protein is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving color reaction with a suitable substrate, and

C) complement protein and a further specific anti-complement antibody, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate;

(c) when the antibody or complement protein of step (b) is conjugated with an enzyme, incubating said device with a solution of said suitable enzyme substrate; and

(d) detecting the signal of said indicator of step (b).

23. The method of claim 22, wherein the incubation with a sample containing the immunoglobulin or antigen to be detected is in the presence of non-specific proteins with regard to the immunoreactive compounds.

24. The method of claim 22, wherein the indicator is selected from the group consisting of radioactively labelled antibodies, antibodies conjugated with a fluorescent substance, and antibodies conjugated with an enzyme capable of giving a color reaction with a suitable substrate.

25. A method of claim 22, wherein the indicator is a horseradish peroxidase conjugated antibody.

26. An immunological analysis device consisting of a porous sheet of nitrocellulose containing an array of preselected geometry of delimited absorption areas of at least one antigen, which adheres tightly and does not spread out on the surface of said porous sheet; said array of preselected geometry being obtained in said porous sheet by applying liquid aliquots of said antigen to said porous sheet mechanically via direct contact, wherein residual absorption sites in said porous sheet are unsaturated or saturated by non-specific protein.

27. The device of claim 26 wherein said porous sheet is from about 0.01 to about 0.5 mm thick.

28. The device of claim 27 wherein said porous sheet is about 0.1 mm thick.

29. The device of claim 27 wherein said nitrocellulose has about 3 nitric acid groups per 6 carbon atoms.

30. The device of claim 27 wherein said porous sheet has an average pore size of about 0.45  $\mu\text{m}$ .

31. The device of claim 27 wherein said array is an array of dots.

32. The device of claim 27 wherein said array is an array of microdots having diameters less than 2 mm.

33. The device of claim 27 wherein said array is an array of lines of width of 2 mm or less.

34. The device of claim 26 wherein said antigen is applied to said support by a capillary tube, pipette, syringe, or spray.

35. The device of claim 26 wherein said antigen is applied to said support in volumes of less than 1 ul.

36. The device of claim 26 wherein said residual adsorption area is saturated with a serum containing substance, wherein said substance is undiluted total serum a total serum diluted with sodium chloride.

37. The device of claim 26 wherein said antigen is a constituent of human biopsy material, mammalian tissue, mammalian cells, body fluids, fungi, protozoa, metazoan parasites, bacteria, mycoplasma, or viruses.

38. A kit comprising the device of claim 26 and reagents of an indicator system in pre-aliquoted or dessicated form to allow detection of an antigen -- antigen reactive compound complex.

39. The kit of claim 38 wherein said indicator is selected from the group consisting

A) antibodies capable of forming antigen-antibody complexes, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate,

B) complement protein capable of binding to antigen-antibody complexes, wherein the complement protein is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving color reaction with a suitable substrate, and

C) complement protein and a further specific anti-complement antibody, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate.

40. The kit of claim 38, comprising a multi-cavity plastic tray, lyophilized mixtures of indicator antibody, salts, buffers, carrier serum or protein, and pre-determined amounts of indicator enzyme chromogenic substrate, salts, buffers, and ampoules containing pre-measured volumes of liquid substrates, all in suitable packaging.

41. The kit of claim 38 wherein said reagents of said indicator system comprise a substrate, a cofactor or prosthetic group for an indicator enzyme, or a coupled series of enzymes.

42. The kit of claim 38, wherein the reagents of said indicator system comprise a covalent adduct between an antigen and a signalling molecule.

43. A method of immunological analysis comprising (a) incubating the device of claim 26 wherein residual absorption sites in said porous sheet are saturated by non-specific protein, with a sample containing an immunoglobulin to be detected;

(b) incubating said device with a solution of an indicator selected from the group consisting of

A) antibodies capable of forming antigen-antibody complexes, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate,

B) complement protein capable of binding to antigen-antibody complexes, wherein the complement protein is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving color reaction with a suitable substrate, and

C) complement protein and a further specific anti-complement antibody, wherein the antibody is radioactively labelled, conjugated with a fluorescent substance or conjugated with an enzyme capable of giving a color reaction with a suitable substrate;

(c) when the antibody or complement protein of step (b) is conjugated with an enzyme, incubating said device with a solution of said suitable enzyme substrate; and

d) detecting the signal of said indicator of step (b).

### Status: Path 1 of [Dialog]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID dialog.com)  
Trying 3106...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 00.12.12D

Last logoff: 25jan01 12:32:15

Logon file001 25jan01 15:37:22

NOTICE set ON to \$20.00

You will be prompted to confirm each TYPE or PRINT request where  
format charges exceeds \$20.00

Please enter SUBACCOUNT name/number:

?3776-010140 LaunchCyte BEJ

Is 3776-010140 LAUNCHCYTE BEJ the SUBACCOUNT you want to use? (Y/N)

?y

Subaccount is set to 3776-010140 LAUNCHCYTE BEJ

\*\*\* NEW Current Year Ranges Install \*\*\*

File 1:ERIC 1966-2001/Jan 16

(c) format only 2001 The Dialog Corporation

Set Items Description

-----

Terminal set to DLINK

?b 411

25jan01 15:38:40 User026066 Session D6238.1

Sub account: 3776-010140 LAUNCHCYTE BEJ

\$0.19 0.056 DialUnits File1

\$0.19 Estimated cost File1

\$0.40 TELNET

\$0.59 Estimated cost this search

\$0.59 Estimated total session cost 0.056 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)

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\*\*\* DIALINDEX search results display in an abbreviated \*\*\*

\*\*\* format unless you enter the SET DETAIL ON command. \*\*\*

?(microarray? or micro(w2)array? or chip? or biochip?) and (protein? or peptid? or substrat?) and enzym?

>>>(" command not valid in DIALINDEX.

?s (microarray? or array? or chip? or biochip?) and (protein? or peptid? or substrat?) and enzym?

>>>No files selected. Use SET FILES to choose at least two files; then use  
SELECT alone to reissue this SELECT statement.

?sf all

You have 576 files in your file list.

(To see banners, use SHOW FILES command)

?select

Your SELECT statement is:

s (microarray? or array? or chip? or biochip?) and (protein? or peptid?  
or substrat?) and enzym?

Items	File
1	1: ERIC_1966-2001/Jan 16
84	2: INSPEC_1969-2001/Jan W3
1164	5: Biosis Previews(R)_1969-2001/Jan W4
31	6: NTIS_1964-2001/Feb W1
4	7: Social SciSearch(R)_1972-2001/Jan W3
82	8: Ei Compendex(R)_1970-2000/Dec W5
90	9: Business & Industry(R)_Jul/1994-2001/Jan 24
91	10: AGRICOLA_70-2001/Jan
1	11: PsycINFO(R)_1887-2001/Jan W3
9	13: BAMP_2001/Jan W2
1	14: Mechanical Engineering Abs_1973-2001/Jan
137	15: ABI/Inform(R)_1971-2001/Jan 25
410	16: Gale Group PROMT(R)_1990-2001/Jan 24
5	18: Gale Group F&S Index(R)_1988-2001/Jan 24
9	19: CHEM.INDUSTRY NOTES_1974-2001/ISS 200104
145	20: World Reporter_1997-2001/Jan 25
4	28: Oceanic Abst._1964-2001/Jan
1	29: Meteor.& Geoastro.Abs._1970-2001/Feb
5	30: AsiaPacific_1985-2001/Jan 10
1009	34: SciSearch(R)_Cited Ref_Sci_1990-2001/Jan W3
159	35: Dissertation Abstracts Online_1861-2000/Dec
6	40: Enviroline(R)_1975-2001/Jan
10	41: Pollution Abs_1970-2001/Jan
27	44: Aquatic Sci&Fish Abs_1978-2001/Jan
920	47: Gale Group Magazine DB(TM)_1959-2001/Jan 24
184	50: CAB Abstracts_1972-2001/Dec
47	51: Food Sci.&Tech.Abs_1969-2001/Mar W4
17	53: FOODLINE(R): Food Science & Technology_1972-2001/Jan 24
6	62: SPIN(R)_1975-2000/Nov W4
Examined 50	files
1	67: World Textiles_1968-2001/Jan
2	68: Env.Bib._1974-2000/Oct
9	70: SEDBASE_1996/Jan Q1

### Status: Break Sent.

?s (microarray? or chip? or biochip?) and (protein? or peptid? or substrat?) and enzym?  
and (aerosol? or mist? or fog?)

Your SELECT statement is:

s (microarray? or chip? or biochip?) and (protein? or peptid? or  
substrat?) and enzym? and (aerosol? or mist? or fog?)

Items	File
1	9: Business & Industry(R)_Jul/1994-2001/Jan 24
19	15: ABI/Inform(R)_1971-2001/Jan 25
11	16: Gale Group PROMT(R)_1990-2001/Jan 24
4	20: World Reporter_1997-2001/Jan 25
39	47: Gale Group Magazine DB(TM)_1959-2001/Jan 24
Examined 50	files
1	75: TGG Management Contents(R)_86-2001/Jan W1
50	88: Gale Group Business A.R.T.S._1976-2001/Jan 23
10	98: General Sci Abs/Full-Text_1984-2001/Dec
1	112: UBM Industry News_1998-2001/Jan 25
Examined 100	files
1	129: PHIND(Archival)_1980-2001/Jan W2
14	141: Readers Guide_1983-2001/Dec
4	146: Washington Post Online_1983-2001/Jan 23
59	148: Gale Group Trade & Industry DB_1976-2001/Jan 24
32	149: TGG Health&Wellness DB(SM)_1976-2001/Jan W2
1	158: DIOGENES(R)_1976-2001/Jan W1
35	180: Federal Register_1985-2001/Jan 25
Examined 150	files
2	229: Drug Info._2000/Q3
Examined 200	files



2 262: CBCA Fulltext\_1982-2001/Jan  
1 275: Gale Group Computer DB(TM)\_1983-2001/Jan 22

### Status: Break Sent.

?s (microarray? or chip? or biochip?) and (protein? or peptid? or substrat?) and enzym?  
and (aerosol? or mist? or fog? or atomiz?) and scan?

Your SELECT statement is:

s (microarray? or chip? or biochip?) and (protein? or peptid? or  
substrat?) and enzym? and (aerosol? or mist? or fog? or atomiz?) and scan?

Items	File
8	15: ABI/Inform(R)_1971-2001/Jan 25
4	16: Gale Group PROMT(R)_1990-2001/Jan 24
17	47: Gale Group Magazine DB(TM)_1959-2001/Jan 24
Examined 50 files	
1	75: TGG Management Contents(R)_86-2001/Jan W1
24	88: Gale Group Business A.R.T.S._1976-2001/Jan 23
4	98: General Sci Abs/Full-Text_1984-2001/Dec
1	112: UBM Industry News_1998-2001/Jan 25
Examined 100 files	
1	129: PHIND(Archival)_1980-2001/Jan W2
3	141: Readers Guide_1983-2001/Dec
30	148: Gale Group Trade & Industry DB_1976-2001/Jan 24
12	149: TGG Health&Wellness DB(SM)_1976-2001/Jan W2
23	180: Federal Register_1985-2001/Jan 25
Examined 150 files	
1	229: Drug Info._2000/Q3
Examined 200 files	
2	262: CBCA Fulltext_1982-2001/Jan
Examined 250 files	
45	348: EUROPEAN PATENTS_1978-2000/Jan W02

Processing

>>>File 349 processing for PROTEIN? stopped at PROTEINSTRUKUREN

>>>File 349 processing for PEPTID? stopped at PEPTIDYLPHOSPHONATES

513	349: PCT Fulltext_1983-2001/UB=20010118, UT=20010104
1	370: Science_1996-1999/Jul W3
4	388: PEDS: Defense Program Summaries_1999/May
Examined 300 files	
1	440: Current Contents Search(R)_1990-2001/Feb W1
2	442: AMA Journals_1982-2000/Oct B3
2	444: New England Journal of Med._1985-2001/Jan W4
1	457: The Lancet_1986-2000/Oct W1
14	484: Periodical Abstracts Plustext_1986-2001/Jan W3
Examined 350 files	
1	553: Wilson Bus. Abs. FullText_1982-2001/Dec
Examined 400 files	
1	587: Jane's Defense&Aerospace_2001/Jan W2
5	590: KOMPASS Western Europe_2000/Sep
1	634: San Jose Mercury_Jun 1985-2001/Jan 23
3	636: Gale Group Newsletter DB(TM)_1987-2001/Jan 24
Examined 450 files	
9	653: US Patents Fulltext_1980-1989

Processing

129	654: US Pat.Full._1990-2001/Jan 23
5	660: Federal News Service_1991-2001/Jan 25
1	707: The Seattle Times_1989-2001/Jan 22
Examined 500 files	
Examined 550 files	
1	810: Business Wire_1986-1999/Feb 28

33 files have one or more items; file list includes 576 files.  
One or more terms were invalid in 3 files.

?b 15,16,47,75,88,98,112,129,141,148,149,229,262,348,370,388,440,442,444,457,484,553,58  
7,590,634,636,653,654,660,707,810

25jan01 15:59:20 UserC26056 Session D6238.2

Sub account: 3776-010140 LAUNCHCYTE BEJ  
\$30.43 24.344 DialUnits File411  
\$30.43 Estimated cost File411  
\$4.20 TELNET  
\$34.63 Estimated cost this search  
\$35.22 Estimated total session cost 24.399 DialUnits

SYSTEM:OS - DIALOG OneSearch

File 15:ABI/Inform(R) 1971-2001/Jan 25  
(c) 2001 Bell & Howell  
File 16:Gale Group PROMT(R) 1990-2001/Jan 24  
(c) 2001 The Gale Group  
File 47:Gale Group Magazine DB(TM) 1959-2001/Jan 24  
(c) 2001 The Gale group  
File 75:TGG Management Contents(R) 86-2001/Jan W1  
(c) 2001 The Gale Group  
File 88:Gale Group Business A.R.T.S. 1976-2001/Jan 23  
(c) 2001 The Gale Group  
File 98:General Sci Abs/Full-Text 1984-2001/Dec  
(c) 2001 The HW Wilson Co.  
File 112:UBM Industry News 1998-2001/Jan 25  
(c) 2001 United Business Media  
File 129:PHIND(Archival) 1980-2001/Jan W2  
(c) 2001 PJB Publications, Ltd.  
**\*File 129: Please note new price changes effective January 1, 2001.**  
See Help Rates129 for details.  
File 141:Readers Guide 1983-2001/Dec  
(c) 2001 The HW Wilson Co  
File 148:Gale Group Trade & Industry DB 1976-2001/Jan 24  
(c)2001 The Gale Group  
File 149:TGG Health&Wellness DB(SM) 1976-2001/Jan W2  
(c) 2001 The Gale Group  
File 229:Drug Info. 2000/Q3  
(c) 2000 Amer.Soc.of Health-Systems Pharm.  
**\*File 229: This file has been reloaded. Accession Numbers have changed.**  
File 262:CBCA Fulltext 1982-2001/Jan  
(c) 2001 Micromedia Ltd.  
File 348:EUROPEAN PATENTS 1978-2000/Jan W02  
(c) 2001 European Patent Office  
File 370:Science 1996-1999/Jul W3  
(c) 1999 AAAS  
File 388:PEDS: Defense Program Summaries 1999/May  
(c) 1999 Forecast Intl/DMS  
**\*File 388: PEDS is a closed file (will no longer update).**  
File 440:Current Contents Search(R) 1990-2001/Feb W1  
(c) 2001 Inst for Sci Info  
**\*File 440: Please note new price changes effective January 1, 2001.**  
See Help Rates440 for details.  
File 442:AMA Journals 1982-2000/Oct B3  
(c)2000 Amer Med Assn -FARS/DARS apply  
**\*File 442: There is no data missing. UDs have been adjusted to reflect the current months data. See Help News442 for details.**  
File 444:New England Journal of Med. 1985-2001/Jan W4  
(c) 2001 Mass. Med. Soc.  
File 457:The Lancet 1986-2000/Oct W1  
(c) 2000 The Lancet, Ltd.  
**\*File 457: Due to production changes at The Lancet, the updating of this file is delayed.**  
File 484:Periodical Abstracts Plustext 1986-2001/Jan W3  
(c) 2001 Bell & Howell  
File 553:Wilson Bus. Abs. FullText 1982-2001/Dec  
(c) 2001 The HW Wilson Co  
File 587:Jane's Defense&Aerospace 2001/Jan W2  
(c) 2001 JANE'S INFORMATION GROUP  
File 590:KOMPASS Western Europe 2000/Sep  
(c) 2000 KOMPASS Intl.  
File 634:San Jose Mercury Jun 1985-2001/Jan 23  
c 2001 San Jose Mercury News

File 636:Gale Group Newsletter DB(TM) 1987-2001/Jan 24

(c) 2001 The Gale Group

File 653:US Patents Fulltext 1980-1989

(c) format only 2001 The Dialog Corp.

\*File 653: Reassignment data current through 7/25/2000 recordings.

Due to recent processing problems, the SORT command is not working.

File 654:US Pat.Full. 1990-2001/Jan 23

(c) format only 2001 The Dialog Corp.

\*File 654: Reassignment data current through 12/5/2000 recordings.

File 660:Federal News Service 1991-2001/Jan 25

(c) 2001 Federal News Service

\*File 660: Daily updates resumed January 28, 2000. Missing stories

are available from October 18, 1999. See HELP NEWS 660 for more info.

File 707:The Seattle Times 1989-2001/Jan 22

(c) 2001 Seattle Times

File 810:Business Wire 1986-1999/Feb 28

(c) 1999 Business Wire

Set Items Description

---  
?s (microarray? or chip? or biochip?) and (protein? or peptid? or substrat?) and enzym?  
and (aerosol? or mist? or fog? or atomiz?) and scan?

Processing

Processed 10 of 31 files ...

Processing

Processing

Processed 20 of 31 files ...

Processing

Processed 30 of 31 files ...

Completed processing all files

4716 MICROARRAY?

1095418 CHIP?

2067 BIOCHIP?

1459304 PROTEIN?

346284 PEPTID?

760040 SUBSTRAT?

673405 ENZYM?

116687 AEROSOL?

759660 MIST?

166160 FOG?

46249 ATOMIZ?

1347081 SCAN?

S1 334 (MICROARRAY? OR CHIP? OR BIOCHIP?) AND (PROTEIN? OR  
PEPTID? OR SUBSTRAT?) AND ENZYM? AND (AEROSOL? OR MIST?  
OR FOG? OR ATOMIZ?) AND SCAN?

?print 1/5/1-334

Estimated cost of output requested is: \$1065.10

Do you want to proceed with this PRINT request? Y = Yes N = No

(Enter N if you are saving an Alert profile; the current PRINT will be cancelled.)

?n

PRINT Command cancelled.

?print 1/4/1-334

>>>FORMAT '04' not found in file 634.

>>>FORMAT '04' not found in file 707.

Estimated cost of output requested is: \$822.35

Do you want to proceed with this PRINT request? Y = Yes N = No

(Enter N if you are saving an Alert profile; the current PRINT will be cancelled.)

?n

PRINT Command cancelled.

?t 1/3/1

1/3/1 (Item 1 from file: 15)

DIALOG(R)File 15:ABI/Inform(R)

(c) 2001 Bell & Howell. All rts. reserv.

01973374 48023028

Part 1: Apollo meets minerva: Health in 2025

J, Wanda  
Health Forum Journal v43n1 PP: 22-29 Jan/Feb 2000  
JRNL CODE: HPF  
WORD COUNT: 6027  
?t 1/6/1

1/6/1 (Item 1 from file: 15)  
01973374 48023028

**\*\*USE FORMAT 9 FOR FULL TEXT\*\***

Part 1: Apollo meets minerva: Health in 2025  
Jan/Feb 2000 LENGTH: 7 Pages  
WORD COUNT: 6027

?t 1/4/1

>>>Format 4 is not valid in file 634

>>>Format 4 is not valid in file 707

1/4/1 (Item 1 from file: 15)  
DIALOG(R)File 15:ABI/Inform(R)  
(c) 2001 Bell & Howell. All rts. reserv.

01973374 48023028

Part 1: Apollo meets minerva: Health in 2025

J, Wanda

Health Forum Journal v43n1 PP: 22-29 Jan/Feb 2000 JRNL CODE: HPF  
DOC TYPE: Periodical; Feature LANGUAGE: English RECORD TYPE: Fulltext  
LENGTH: 7 Pages  
WORD COUNT: 6027

ABSTRACT: Predictions on the state of the health care industry in 2025 are presented.

?print 1/5/1-334

Estimated cost of output requested is: \$1065.10  
Do you want to proceed with this PRINT request? Y = Yes N = No  
(Enter N if you are saving an Alert profile; the current PRINT will be cancelled.)  
?y

P056: PRINT 1/5/1-334 est. cost of \$1065.10  
Estimated postal surcharge (\$0.10 per record per copy) is \$33.40.

**\*\*\* NOTE: Print cancellation window has been reduced to 30 minutes.**  
For more information, enter HELP CANCEL.

?b 349

25jan01 16:04:03 User026066 Session D6238.3

Sub account: 3776-010140 LAUNCHCYTE BEJ

\$0.59 0.110 DialUnits File15

\$1.55 1 Type(s) in Format 3

\$3.20 1 Type(s) in Format 4

\$0.00 1 Type(s) in Format 6

\$4.75 3 Types

\$25.60 8 Print(s) in Format 5

\$25.60 1 Print transaction(s)

\$30.94 Estimated cost File15

\$1.20 0.222 DialUnits File16

\$13.00 4 Print(s) in Format 5

\$13.00 1 Print transaction(s)

\$14.20 Estimated cost File16

\$0.79 0.145 DialUnits File47

\$36.55 17 Print(s) in Format 5

\$36.55 1 Print transaction(s)

\$37.34 Estimated cost File47

\$0.16 0.037 DialUnits File75

\$2.60 1 Print(s) in Format 5

\$2.60 1 Print transaction(s)

\$2.76 Estimated cost File75

\$0.70 0.158 DialUnits File88

\$51.60 24 Print(s) in Format 5

\$51.60 1 Print transaction(s)

\$52.30 Estimated cost File88  
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     \$6.20   1 Print transaction(s)  
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 \$1.28 Estimated cost File112  
     \$1.46     0.057 DialUnits File129  
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     \$4.80   1 Print transaction(s)  
 \$6.26 Estimated cost File129  
     \$0.14     0.058 DialUnits File141  
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     \$4.20   1 Print transaction(s)  
 \$4.34 Estimated cost File141  
     \$1.10     0.203 DialUnits File148  
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 \$82.10 Estimated cost File148  
     \$0.31     0.070 DialUnits File149  
         \$25.80   12 Print(s) in Format   5  
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 \$26.11 Estimated cost File149  
     \$0.04     0.015 DialUnits File229  
         \$7.80   1 Print(s) in Format   5  
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 \$7.84 Estimated cost File229  
     \$0.33     0.058 DialUnits File262  
         \$4.20   2 Print(s) in Format   5  
     \$4.20   1 Print transaction(s)  
 \$4.53 Estimated cost File262  
     \$2.41     0.529 DialUnits File348  
         \$240.75   45 Print(s) in Format   5  
     \$240.75   1 Print transaction(s)  
 \$243.16 Estimated cost File348  
     \$0.12     0.033 DialUnits File370  
         \$1.40   1 Print(s) in Format   5  
     \$1.40   1 Print transaction(s)  
 \$1.52 Estimated cost File370  
     \$0.10     0.025 DialUnits File388  
         \$88.20   4 Print(s) in Format   5  
     \$88.20   1 Print transaction(s)  
 \$88.30 Estimated cost File388  
     \$4.51     0.315 DialUnits File440  
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     \$0.14     0.031 DialUnits File442  
         \$8.90   2 Print(s) in Format   5  
     \$8.90   1 Print transaction(s)  
 \$9.04 Estimated cost File442  
     \$0.11     0.022 DialUnits File444  
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     \$8.90   1 Print transaction(s)  
 \$9.01 Estimated cost File444  
     \$0.19     0.039 DialUnits File457  
         \$2.15   1 Print(s) in Format   5  
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     \$0.58     0.118 DialUnits File484  
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     \$0.09     0.039 DialUnits File553  
         \$2.55   1 Print(s) in Format   5  
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 \$2.64 Estimated cost File553

\$0.09 0.016 DialUnits File587  
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 \$3.20 1 Print transaction(s)  
 \$3.29 Estimated cost File587  
 \$0.31 0.048 DialUnits File590  
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 \$14.00 1 Print transaction(s)  
 \$14.31 Estimated cost File590  
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 \$1.55 1 Print transaction(s)  
 \$1.58 Estimated cost File634  
 \$0.34 0.064 DialUnits File636  
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 \$9.75 1 Print transaction(s)  
 \$10.09 Estimated cost File636  
 \$1.04 0.176 DialUnits File653  
 \$24.30 9 Print(s) in Format 5  
 \$24.30 1 Print transaction(s)  
 \$25.34 Estimated cost File653  
 \$3.67 0.622 DialUnits File654  
 \$348.30 129 Print(s) in Format 5  
 \$348.30 1 Print transaction(s)  
 \$351.97 Estimated cost File654  
 \$0.02 0.020 DialUnits File660  
 \$7.75 5 Print(s) in Format 5  
 \$7.75 1 Print transaction(s)  
 \$7.77 Estimated cost File660  
 \$0.05 0.050 DialUnits File707  
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 \$1.55 1 Print transaction(s)  
 \$1.60 Estimated cost File707  
 \$0.03 0.028 DialUnits File810  
 \$2.60 1 Print(s) in Format 5  
 \$2.60 1 Print transaction(s)  
 \$2.63 Estimated cost File810  
 OneSearch, 31 files, 3.463 DialUnits FileOS  
 \$1.00 TELNET  
 \$1,091.88 Estimated cost this search  
 \$1,127.10 Estimated total session cost 27.862 DialUnits

File 349:PCT Fulltext 1983-2001/UB=20010118, UT=20010104  
 (c) 2001 WIPO/MicroPat

\*File 349: Phase 2 enhancements with current WIPO biblio data now online.  
 See HELP NEWS 349 for more information.

Set Items Description

?s (microarray? or chip? or biochip?) and (protein? or peptid? or substrat?) and enzym?  
 and (aerosol? or mist? or fog? or atomiz?) and scan? and (enzym?(w3)react? or enzym?(w  
 3)assay?)  
 >>>File 349 processing for PROTEIN? stopped at PROTEINSTRUKUREN  
 >>>File 349 processing for PEPTID? stopped at PEPTIDYLPHOSPHONATES  
 >>>File 349 processing for REACT? stopped at REACTSINTS  
 Processing

731 MICROARRAY?  
 35060 CHIP?  
 190 BIOCHIP?  
 69931 PROTEIN?  
 47283 PEPTID?  
 88172 SUBSTRAT?  
 62658 ENZYM?  
 17111 AEROSOL?  
 8837 MIST?  
 3903 FOG?  
 5268 ATOMIZ?  
 52222 SCAN?  
 52658 ENZYM?

155242 REACT?  
8191 ENZYM?(3W) REACT?  
62658 ENZYM?  
52583 ASSAY?  
11209 ENZYM?(3W) ASSAY?  
S1 381 (MICROARRAY? OR CHIP? OR BIOCHIP?) AND (PROTEIN? OR  
PEPTID? OR SUBSTRAT?) AND ENZYM? AND (AEROSOL? OR MIST?  
OR FOG? OR ATOMIZ?) AND SCAN? AND (ENZYM?(W3) REACT? OR  
ENZYM?(W3) ASSAY?)

?t 1/5/1

1/5/1  
DIALOG(R) File 349:PCT Fulltext  
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00768510

**NOVEL KINASES AND USES THEREOF**

**NOUVELLES KINASES ET UTILISATIONS DE CES DERNIERES**

Patent Applicant/Assignee:

MILLENNIUM PHARMACEUTICALS INC, 75 Sydney Street, Cambridge, MA 02139, US  
, US (Residence), US (Nationality)

Inventor(s):

HODGE Martin R, 39 Crawford Street, Arlington, MA 02474, US

MEYERS Rachel, 75 Sidney Street, Cambridge, MA 02139, US

WILLIAMSON Mark, 15 Stonecrest Drive, Saugus, MA 01906, US

Legal Representative:

SPRUIELL W Murray, Alston & Bird LLP, P.O. Drawer 34009, Charlotte, NC  
28234-4009, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200100879 A1 20010104 (WO 0100879)

Application: WO 2000US18291 20000630 (PCT/WO US0018291)

Priority Application: US 99345473 19990630; US 2000562480 20000501

Designated States: AE AG AL AM AT AT (utility model) AU AZ BA BB BG BR BY  
BZ CA CH CN CR CU CZ CZ (utility model) DE DE (utility model) DK DK  
(utility model) DM DZ EE EE (utility model) ES FI FI (utility model) GB  
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA  
MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SK (utility model)  
SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12Q-001/68

International Patent Class: C12Q-001/48; C12N-001/20; C12N-015/00;

C12N-005/00; C12N-009/12; C07H-021/04; A61K-039/00; G01N-033/00;

G01N-033/53; C07K-016/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 32580

**English Abstract**

Novel kinase **polypeptides**, **proteins**, and nucleic acid molecules are disclosed. In addition to isolated, full-length kinase **proteins**, the invention further provides isolated kinase fusion **proteins**, antigenic **peptides**, and anti-kinase antibodies. The invention also provides kinase nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a kinase gene has been introduced or disrupted. Diagnostic, screening, and therapeutic methods utilizing compositions of the invention are also provided.

**French Abstract**

On decrit de nouveaux **polypeptides**, de nouvelles **proteines** et de nouvelles molecules d'acide nucleique de kinase. Hormis des **proteines** kinase isolees, entieres, la presente invention concerne egalement des

proteines hybrides kinase, des peptides antigeniques et des anticorps anti-kinase. L'invention concerne egalement des molecules d'acide nucleique kinase, des vecteurs d'expression de recombinaison contenant une molecule d'acide nucleique selon la presente invention, des cellules hotes dans lesquelles les vecteurs d'expression ont ete introduits et des animaux transgeniques non humains dans lesquels un gene kinase a ete introduit ou perturbe. On decrit egalement des procedes de diagnostic, de criblage et de traitement dans lesquels on utilise les compositions selon la presente invention.

Legal Status (Type, Date, Text)

Publication 20010104 A1 With international search report.

?s s1 and (microarray?/ab or chip?/ab or biochip?/ab)

381 S1

31 MICROARRAY?/AB

4112 CHIP?/AB

23 BIOCHIP?/AB

S2

4 S1 AND (MICROARRAY?/AB OR CHIP?/AB OR BIOCHIP?/AB)

?t 2/5/1-4

Estimated cost of output requested is: \$20.40

Are you ready to receive all output? (Yes/No/Help)

?y

2/5/1

DIALOG(R) File 349:PCT Fulltext

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00713633

NUCLEIC ACID ANALYSIS USING SEQUENCE-TARGETED TANDEM HYBRIDIZATION

ANALYSE D'ACIDES NUCLEIQUES PAR HYBRIDATION EN TANDEM CIBLEE SUR DES SEQUENCES

Patent Applicant/Inventor:

BEATTIE Kenneth Loren, 234 Open Range Road, Crossville, TN 38555, US,  
US (Residence), US (Nationality)

MALDONADO RODRIGUEZ Rogelio, Cerrada Merced de las Huertas #28, Mexico  
City, D.F. 11420, MX, MX (Residence), MX (Nationality)

Legal Representative:

ADLER Benjamin A, McGregor & Adler, 8011 Candle Lane, Houston, TX 77071,  
US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200026412 A1 20000511 (WO 0026412)

Application: WO 99US25693 19991102 (PCT/WO US9925693)

Priority Application: US 98106655 19981102

Designated States: CA JP MX

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: C12Q-001/68

International Patent Class: C12P-019/34; G01N-033/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 22669

English Abstract

The disclosed invention provides a novel method for analyzing genomic DNA and expressed sequences using auxiliary oligonucleotides, preannealed to the single-stranded target nucleic acid to form a partially duplex target molecule, offers several advantages in the analysis of nucleic acid sequences by hybridization to genosensor arrays or "DNA chips". Also provided is a method for directly analyzing and comparing patterns of gene expression at the level of transcription in different cellular samples.

French Abstract

La presente invention concerne une nouvelle technique d'analyse de l'ADN genomique et des sequences exprimees, qui utilise des oligonucleotides auxiliaires, prealablement anneles avec l'acide nucleique monocatenaire



cible pour former une molecule cible partiellement duplex. L'invention presente plusieurs avantages dans l'analyse de sequences d'acide nucleique par hybridation avec des matrices de "genocapteurs" ou puces a ADN. L'invention concerne egalement un procede permettant d'analyser et de comparer directement des modeles d'expression genique au niveau de la transcription dans differents echantillons cellulaires.

Legal Status (Type, Date, Text)

Publication 20000511 A1 With international search report.

Examination 20000928 Request for preliminary examination prior to end of 19th month from priority date

2/5/2

DIALOG(R) File 349:PCT Fulltext

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00678691 \*\*Image available\*\*

**MICROFABRICATED CELL SORTER**

**TRIEUR DE CELLULES MICROFABRIQUE**

Patent Applicant/Assignee:

CALIFORNIA INSTITUTE OF TECHNOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY ,  
1200 East California Boulevard, Pasadena, CA 91125 , US

Inventor(s):

QUAKE Stephen, QUAKE, Stephen , San Marino, CA , US

FU Anne, FU, Anne , Hacienda Hills, CA , US

ARNOLD Frances, ARNOLD, Frances , 629 S. Grand Avenue, Pasadena, CA 91105  
, US

SPENCE Charles F, SPENCE, Charles, F. , Arcadia, CA , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9961888 A2 19991202

Application: WO 99US13050 19990521 (PCT/WO US9913050)

Priority Application: US 9886394 19980522; US 98108894 19981117

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE

ES FI GB GD GE HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV

MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG

US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ

TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI

CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: G01N-015/14;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23518

English Abstract

The invention provides a microfabricated device for sorting cells based on a desired characteristic, for example, reporter-labeled cells can be sorted by the presence or level of reporter on the cells. The device includes a chip having a substrate into which is microfabricated at least one analysis unit. Each analysis unit includes a main channel, having a sample inlet channel, typically at one end, and a detection region along a portion of its length. Adjacent and downstream from the detection region, the main channel has a discrimination region or branch point leading to at least two branch channels. The analysis unit may further include additional inlet channels, detection points, branch points, and branch channels as desired. A stream containing cells is passed through the detection region, such that on average one cell occupies the detection region at a given time. The cells can be sorted into an appropriate branch channel based on the presence or amount of a detectable signal such as an optical signal, with or without stimulation, such as exposure to light in order to promote fluorescence.

French Abstract

L'invention concerne un dispositif microfabrique permettant de trier des cellules en fonction d'une caracteristique souhaitee. Des cellules marquées par un marqueur peuvent par exemple etre trieées en fonction de

la presence ou de la quantite d'un marqueur sur ces cellules. Le dispositif de cette invention comprend une puce pourvue d'un substrat a l'interieur duquel est microfabriquee au moins une unite d'analyse. Chaque unite d'analyse comprend un canal principal renfermant un canal d'admission d'echantillon, generalement place a une extremite, et une zone de detection situee le long d'une partie de la longueur de ladite unite d'analyse. A proximite de cette zone de detection, et en aval de celle-ci, ledit canal principal renferme une zone de separation ou un point de ramification menant a deux canaux de ramification. Cette unite d'analyse peut egalement comprendre autant de canaux d'admission, de points de detection, de points de ramification, et de canaux de ramification que necessaire. Un courant renfermant des cellules traverse ladite zone de detection, de sorte qu'en moyenne, une cellule occupe cette zone de detection a un moment donne. Les cellules peuvent donc etre trieess dans un canal de ramification approprie, en fonction de la presence ou de la quantite d'un signal detectable comme un signal optique, et ce avec ou sans stimulation telle qu'une exposition a la lumiere destinee a favoriser la fluorescence.

Legal Status (Type, Date, Text)

Correction 20000602 Corrected version of Pamphlet: pages 1/15-15/15, drawings, replaced by new pages 1/14-14/14; due to late transmittal by the receiving Office

2/5/3

DIALOG(R) File 349:PCT Fulltext

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00643938 \*\*Image available\*\*

**INTEGRATED CIRCUIT BIOCHIP MICROSYSTEM**

**MICROSYSTEME SOUS FORME DE BIOPUCE A CIRCUIT INTEGRE**

Patent Applicant/Assignee:

LOCKHEED MARTIN ENERGY RESEARCH CORPORATION, LOCKHEED MARTIN ENERGY RESEARCH CORPORATION, 701 Scarboro Road, P.O. Box 2009, Oak Ridge, TN 37831-8243, US

Inventor(s):

VO-DINH Tuan, VO-DINH, Tuan, 12318 River Oaks Point, Knoxville, TN 37922, US

WINTENBERG Alan, WINTENBERG, Alan, 11521 N. Monticello Drive, Knoxville, TN 37922, US

ERICSON Milton N, ERICSON, Milton, N., 1517 Moorgate Drive, Knoxville, TN 37922, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9927140 A1 19990603

Application: WO 98US25294 19981125 (PCT/WO US9825294)

Priority Application: US 97979672 19971126

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 32983

English Abstract

The present invention discloses a self-contained miniature biosensor designed to detect specific molecular targets, and in particular polypeptides and polynucleotides. For example, hybridized nucleic acids may be detected without external monitoring or signal transmission. The miniaturized biosensor comprises multiple biological sensing elements such as DNA probes, excitation microlasers, a sampling waveguide equipped with optical detectors (fluorescence and Raman), integrated

electro-optics, and a biotelemetric radio frequency signal generator all contained on a single integrated circuit, or "biochip". The novel integrated circuit biochip microsystem (ICBM) is suitable for gene analysis and will allow rapid, large-scale, and cost-effective production of biochips useful in the detection of biological compositions, including the development of multi-array gene chips.

#### French Abstract

La presente invention concerne un biodetecteur miniature monobloc concu pour detecter des molecules cibles specifiques, notamment des polypeptides et des polynucleotides. Par exemple, des acides nucleiques hybrides peuvent etre detectes sans surveillance externe ni transmission de signaux. Le biodetecteur miniaturise comprend plusieurs elements biologiques de detection tels que les sondes d'ADN, des microlasers d'excitation, un guide d'ondes d'echantillonnage equipe de detecteurs optiques (fluorescence et diffusion Raman), des dispositifs electro-optiques, et un generateur de signaux HF biotelemetriques, composants qui sont tous disposes sur un seul circuit integre, ou "biopuce". Le nouveau microsysteme sous forme de biopuce convient a l'analyse genique et permet une production rapide, a grande echelle, et rentable de biopuces utiles pour la detection de compositions biologiques, ainsi que pour la mise au point de puces d'analyse genique multi-voie.

2/5/4

DIALOG(R) File 349:PCT Fulltext

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00613366 \*\*Image available\*\*

ELECTROSPRAYING SOLUTIONS OF SUBSTANCES FOR MASS FABRICATION OF CHIPS AND LIBRARIES

ELECTROVAPORISATION DE SOLUTIONS DE SUBSTANCES POUR LA FABRICATION EN MASSE DE BIOPUCES ET DE BIBLIOTHEQUES DE BIOPUCES

Patent Applicant/Assignee:

NEW YORK UNIVERSITY, NEW YORK UNIVERSITY, 70 Washington Square South,  
New York, NY 10012, US

Inventor(s):

MOROZOV Victor N, MOROZOV, Victor, N., Apartment 3K, 14 Washington  
Place, New York, NY 10003, US

MOROZOVA Tamara Ya, MOROZOVA, Tamara, Ya., Apartment 3K, 14 Washington  
Place, New York, NY 10003, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9858745 A1 19981230

Application: WO 98US12768 19980619 (PCT/WO US9812768)

Priority Application: US 9750274 19970620; US 9755287 19970813

Designated States: AU CA JP NZ US AT BE CH CY DE DK ES FI FR GB GR IE IT LU  
MC NL PT SE

Main International Patent Class: B05B-005/00;

International Patent Class: B05B-005/025; B05B-012/00; B05C-005/02;

B05D-001/04; B05D-001/06; B05D-001/32; B05D-001/36; B05D-003/00;

G01N-021/00; G01N-027/00; G01N-033/50; B01D-059/44;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23563

#### English Abstract

A method of fabricating deposits of non-volatile substances, including biomacromolecules, in the form of spots and films on a substrate surface by electrospray, where the deposits are used to determine the interaction of the deposited non-volatile substances to other substances. Also included in this method is the mass fabrication on a single chip of an array of single and multicomponent microsamples.

#### French Abstract

Cette invention se rapporte a un procede permettant de former des depots de substances non volatiles, notamment de biomacromolecules, sous la

forme de taches et de pellicules sur la surface d'un substrat par  
electrovaporisation, ces depots etant utilises pour determiner  
l'interaction des substances non volatiles deposees avec d'autres  
substances. Ce procede permet egalement la fabrication en masse sur une  
seule biopuce d'un alignement matriciel de micro-echantillons a composant  
unique et a composants multiples.

?logoff

25jan01 16:08:38 User026066 Session D6238.4

Sub account: 3776-010140 LAUNCHCYTE BEJ

\$6.17 1.299 DialUnits File349

\$25.50 5 Type(s) in Format 5

\$25.50 5 Types

\$31.67 Estimated cost File349

\$1.00 TELNET

\$32.67 Estimated cost this search

\$1,159.77 Estimated total session cost 29.161 DialUnits

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### Status: Path 1 of [Dialog]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID dialog.com)  
Trying 3106...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSSS?

### Status: Signing onto Dialog

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ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSSS? \*\*\*\*\*

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Last logoff: 30apr01 10:58:56

Logon file001 30apr01 14:18:04

NOTICE set ON to \$20.00

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?3776-010140-launchcyte-bej

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?y

Subaccount is set to 3776-010140-LAUNCHCYTE-BEJ

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File 1:ERIC 1966-2001/Apr 17

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Set Items Description

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30apr01 14:18:35 User026066 Session D6422.1

Sub account: 3776-010140-LAUNCHCYTE-BEJ

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\$0.20 Estimated cost File1

\$0.10 TELNET

\$0.30 Estimated cost this search

\$0.30 Estimated total session cost 0.059 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)

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\*\*\* DIALINDEX search results display in an abbreviated \*\*\*

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?s (protein? or peptid?) and enzym? and microarray? and (dot? or microdot?) and (slide?  
or chip?)

>>>No files selected. Use SET FILES to choose at least two files; then use  
SELECT alone to reissue this SELECT statement.

?sf all

You have 576 files in your file list.

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?select

Your SELECT statement is:

s (protein? or peptid?) and enzym? and microarray? and (dot? or  
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Items File

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2

16: Gale Group PROMT R\_1990-2001/Apr 27

2 20: World Reporter\_1997-2001/Apr 30  
 Examined 50 files  
 2 88: Gale Group Business A.R.T.S.\_1976-2001/Apr 30  
 2 98: General Sci Abs/Full-Text\_1984-2001/Mar  
 Examined 100 files  
 1 141: Readers Guide\_1983-2001/Mar  
 1 149: TGG Health&Wellness DB(SM)\_1976-2001/Apr W4  
 Examined 150 files  
 Examined 200 files  
 7 348: EUROPEAN PATENTS\_1978-2001/Apr W03  
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 >>>File 349 processing for PEPTID? stopped at PEPTIDYLANTEIL  
 451 349: PCT Fulltext\_1983-2001/UB=20010419, UT=20010405  
 Examined 250 files  
 2 369: New Scientist\_1994-2001/Apr W1  
 Examined 300 files  
 2 440: Current Contents Search(R)\_1990-2001/May W1  
 1 442: AMA Journals\_1982-2001/Apr B2  
 1 444: New England Journal of Med.\_1985-2001/Apr W5  
 1 484: Periodical Abstracts Plustext\_1986-2001/Apr W4  
 Examined 350 files  
 Examined 400 files  
 2 619: Asia Intelligence Wire\_1995-2001/Apr 29  
 2 636: Gale Group Newsletter DB(TM)\_1987-2001/Apr 27  
 Examined 450 files  
 272 654: US PAT.FULL.\_1990-2001/APR 26  
 Examined 500 files  
 1 748: Asia/Pac Bus. Jrnls\_1994-2001/Apr 26  
 Examined 550 files

17 files have one or more items; file list includes 576 files.  
 One or more terms were invalid in 2 files.

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 or chip?) and (three()dimension? or 3()dimension?)  
 Your SELECT statement is:  
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 microdot?) and (slide? or chip?) and (three()dimension? or 3()dimension?)

Items	File
Examined 50 files	
1	98: General Sci Abs/Full-Text_1984-2001/Mar
Examined 100 files	
1	141: Readers Guide_1983-2001/Mar
Examined 150 files	
Examined 200 files	
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>>>File 349 processing for PEPTID? stopped at PEPTIDYLANTEIL	
292	349: PCT Fulltext_1983-2001/UB=20010419, UT=20010405
Examined 250 files	
Examined 300 files	
Examined 350 files	
Processing	
Examined 400 files	
Examined 450 files	
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237	654: US PAT.FULL._1990-2001/APR 26
Examined 500 files	
Examined 550 files	

4 files have one or more items; file list includes 576 files.  
 One or more terms were invalid in 2 files.

?b 98

30apr01 14:39:27 User026066 Session D6422.2  
 Sub account: 3776-010140-LAUNCHCYTE-BEJ  
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\$36.51 Estimated cost File411  
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\$40.71 Estimated cost this search  
\$41.01 Estimated total session cost 29.267 DialUnits

File 98:General Sci Abs/Full-Text 1984-2001/Mar  
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or chip?) and (three()dimension? or 3()dimension?)

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1920 DOT?  
7 MICRODOT?  
1407 SLIDE?  
2486 CHIP?  
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10012 DIMENSION?  
3290 THREE(W)DIMENSION?  
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336 3(W)DIMENSION?

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(DOT? OR MICRODOT?) AND (SLIDE? OR CHIP?) AND  
(THREE()DIMENSION? OR 3()DIMENSION?)

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DIALOG(R)File 98:General Sci Abs/Full-Text  
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04370805 H.W. WILSON RECORD NUMBER: BGSA00120805 (USE FORMAT 7 FOR  
FULLTEXT)

**Beyond the human genome.**

Ezzell, Carol

Scientific American v. 283 no1 (July 2000) p. 64-9

SPECIAL FEATURES: il ISSN: 0036-8733

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

RECORD TYPE: Abstract; Fulltext RECORD STATUS: Corrected or revised  
record

WORD COUNT: 3500

ABSTRACT: Part of a special section on the current state of genomics and the new applied fields that are set to make use of the deciphered human genome. The new industry of proteomics is emerging to capitalize on gene expression and the study of the **proteins** the genes encode. Researchers will soon direct their attention toward the transcriptome, the body of mRNAs produced by a cell at any given time, and the proteome, all the **proteins** manufactured according to the mRNAs' instructions. Determining the precise structural form of each **protein** in the human proteome should aid drug designers in devising chemicals to fit the slots on the **proteins** that either activate them or prevent them from interacting. Some experts estimate that the pharmaceutical industry will, in the next decade, be faced with assessing up to 10,000 human **proteins** against which new therapeutics could be directed, possibly 25 times the total number of drug targets evaluated thus far.

DESCRIPTORS:

Genetic code--Man; Gene expression--Man; DNA chips ; Proteins --  
Analysis

?t 1/7/1

1/7/1

DIALOG(R)File 98:General Sci Abs/Full-Text  
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04370805 H.W. WILSON RECORD NUMBER: BGSA00120805 (THIS IS THE FULLTEXT)  
**Beyond the human genome.**

Ezzell, Carol

Scientific American v. 283 no1 (July 2000) p. 64-9

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

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**ABSTRACT:** Part of a special section on the current state of genomics and the new applied fields that are set to make use of the deciphered human genome. The new industry of proteomics is emerging to capitalize on gene expression and the study of the **proteins** the genes encode. Researchers will soon direct their attention toward the transcriptome, the body of mRNAs produced by a cell at any given time, and the proteome, all the **proteins** manufactured according to the mRNAs' instructions. Determining the precise structural form of each **protein** in the human proteome should aid drug designers in devising chemicals to fit the slots on the **proteins** that either activate them or prevent them from interacting. Some experts estimate that the pharmaceutical industry will, in the next decade, be faced with assessing up to 10,000 human **proteins** against which new therapeutics could be directed, possibly 25 times the total number of drug targets evaluated thus far.

**TEXT:**

Genes are all the rage right now, but in a sense, at this very moment, they are also becoming passe. Now that all the 100,000 or so genes that make up the human genome have been deciphered, a new industry is emerging to capitalize on when and where those genes are active and on identifying and determining the properties of the **proteins** the genes encode. The enterprise, which has so far attracted hundreds of millions of dollars in venture capital and other financing, can be lumped under the newly coined term "proteomics."

"The biggest issue for genomics today is no longer genes," asserts William A. Haseltine, chairman and chief executive officer of Human Genome Sciences in Rockville, Md. "What's interesting is what you do with those genes."

"We have to move on to understand the other elements of the biological process and couple all this information together," agrees Peter Barrett, chief business officer of Celera Genomics, also in Rockville, the company that raced the publicly funded Human Genome Project to sequence the human genome see "The Human Genome Business Today," on page 50. "People took it for granted that the human genome would be done this year. Now it's 'What do we do next?'"

What's next, for the most part, are messenger RNAs (mRNAs) and **proteins**. If DNA is the set of master blueprints a cell uses to construct **proteins**, then mRNA is like the copy of part of the blueprint that a contractor takes to the building site every day. DNA remains in the nucleus of a cell; mRNAs transcribed from active genes leave the nucleus to give the orders for making **proteins**.

Although every cell in the body contains all of the DNA code for making and maintaining a human being, many of those genes are never "turned on," or copied into mRNA, once embryonic development is complete. Various other genes are turned on or off at different times--or not at all--according to the tissue they are in and their role in the body. A pancreatic beta cell, for instance, is generally full of the mRNA instructions for making insulin, whereas a nerve cell in the brain usually isn't.

Scientists used to think that one gene equals one mRNA equals one **protein**, but the reality is much more complicated. They now know that one gene can be read out in portions that are spliced and diced to generate a variety of mRNAs and that subsequent processing of the newly made **proteins** that those transcripts encode can alter their function. The DNA sequence of the human genome therefore tells only a small fraction of the story about what a specific cell is doing. Instead researchers must also pay attention to the transcriptome--the body of mRNAs being produced by a cell



at any given time--and the proteome, all the **proteins** being made according to the instructions in those mRNAs.

#### CASHING IN ON CHIPS

One of the technologies for studying the human transcriptome is the GeneChip system developed by Affymetrix in Santa Clara, Calif. The system is based on thumbnail-size glass **chips** called **microarrays** that are coated with a thin layer of so-called cDNAs, which represent all the mRNAs made by a particular type of cell. (The abbreviation cDNA stands for complementary DNA; it is essentially mRNA artificially translated back into DNA, but without the noncoding sequence gaps, or introns, found in the original genomic DNA.)

To use the system, scientists isolate mRNA from their cellular sample, tag it with a chemical marker and pour it over the **chip**. By observing where the sample mRNA matches and binds to the cDNA on the **chip**, they can identify the mRNA sequences in their sample. Earlier this year Affymetrix launched two new sets of **chips** for analyzing human cell samples. One allows researchers to identify more than 60,000 different human mRNAs; the other can screen cells for roughly 1,700 human mRNAs related to cancer.

The National Cancer Institute in Bethesda, Md., has been examining the mRNAs produced by various types of cancer cells for more than two years now, in a project called the Human Tumor Gene Index. The index is a partnership between government and academic laboratories as well as a group of drug companies that includes BristolMyers Squibb, Genentech, Glaxo Wellcome and Merck. So far they have identified more than 50,000 genes that are active in one or more cancers. For instance, the index has found that 5,692 genes are active in breast cancer cells, including 277 that are not active in other tissues. Compounds that home in on the **proteins** produced by those 277 genes might serve as good cancer drugs with fewer side effects than current chemotherapies. The National Cancer Institute has also recently begun a multi-million-dollar Tissue Proteomics Initiative in conjunction with the U.S. Food and Drug Administration to identify **proteins** involved in cancer.

At bottom, mRNA studies are just a means to better understand the **proteins** in a cell's production line--after all, the **proteins** are the drug targets. And with researchers expecting that the 100,000 or so human genes will turn out to produce more than a million **proteins**, that's a lot of targets. Jean-Francois Formela of Atlas Venture in Boston estimates that within the next decade the pharmaceutical industry will be faced with evaluating up to 10,000 human **proteins** against which new therapeutics might be directed. That's 25 times the number of drug targets that have been evaluated by all pharmaceutical companies since the dawn of the industry, he says.

Mark J. Levin, CEO of Millennium Pharmaceuticals in Cambridge, Mass., says that large pharmaceutical companies, or "big pharma," need to identify between three and five new drug candidates a year in order to grow 10 to 20 percent a year--the minimum increase shareholders will tolerate. "Right now the major pharma companies are only delivering a half to one-and-a-half entities a year," Levin explains. "Their productivity will not sustain their ability to continue to develop and create shareholder value." Millennium has a relationship with Bayer to deliver 225 pretested "druggable" targets within a few years.

"Protein expression is now capturing the imagination of scientists," comments Randall W. Scott, chief scientific officer of Incyte Genomics in Palo Alto, Calif. "It's being able to look not just at a gene and how it's expressed, but at the forms of the **protein**."

#### PROTEIN MACHINES

Scientists at the DNA-sequencing juggernaut Celera are among those getting interested in the study of **protein** expression, or proteomics. Celera has been in negotiations with GeneBio, a commercial adjunct of the Swiss Institute for Bioinformatics in Geneva, to launch a company dedicated to deducing the entire human proteome. Last year Denis F. Hochstrasser, one of the founders of GeneBio, and his colleagues published plans for a molecular scanner that would automate the now tedious process of separating and identifying the thousands of **protein** types in a cell.

The current method for studying **proteins** consists in part of a

technique called two-dimensional gel electrophoresis, which separates **proteins** by charge and size. In the technique, researchers squirt a solution of cell contents onto a narrow polymer strip that has a gradient of acidity. When the strip is exposed to an electric current, each **protein** in the mixture settles into a layer according to its charge. Next, the strip is placed along the edge of a flat gel and exposed to electricity again. As the **proteins** migrate through the gel, they separate according to their molecular weight. What results is a smudgy pattern of **dots**, each of which contains a different **protein**.

In academic laboratories, scientists generally use a tool similar to a hole puncher to cut the **protein** spots from 2-D gels for individual identification by another method, mass spectroscopy see box on opposite page. But the companies Large Scale Biology in Vacaville, Calif., and Oxford GlycoSciences (OGS) in Oxford, England, use robots to do it. OGS is under contract with Pfizer to analyze samples of cerebrospinal fluid taken from patients with various stages of Alzheimer's disease.

The machine devised by Hochstrasser and his research group goes one step further than the robots used by Large Scale Biology and OGS. It would automatically extract the **protein** spots from the gels, use **enzymes** to chop the **proteins** into bits, feed the pieces into a laser mass spectrometer and transfer the information to a computer for analysis. The instrument manufacturer PE Corporation, which owns Celera, has already agreed to make the machines.

With or without robotic arms, 2-D gels have their problems. Besides being tricky to make, they don't resolve highly charged or low-mass **proteins** very well. They also do a poor job of resolving **proteins** with hydrophobic regions, such as those that span the cell membrane. This is a major limitation, because membrane-spanning receptors are important drug targets.

Another method for studying proteomes is what Stephen Oliver of the University of Manchester in England has called "guilt by association": learning about the function of a **protein** by assessing whether it interacts with another **protein** whose role in a cell is known. In February researchers at CuraGen in New Haven, Conn.--together with a group led by Stanley Fields of the Howard Hughes Medical Institute at the University of Washington--reported that they had deduced 957 interactions among 1,004 **proteins** in the baker's yeast *Saccharomyces cerevisiae*. Fields and his colleagues first devised a widely used method for studying **protein** interactions called the yeast two-hybrid system, which uses known **protein** "baits" to find "prey" **proteins** that bind to the "baits" see box on page 67.

The yeast genome has been known to consist of 6,000 genes since it was sequenced in 1996, but the functions of one third of them have remained mysterious. By figuring out which of the unknown **proteins** associated with previously identified ones, the CuraGen and University of Washington scientists were able to sort them into functional categories, such as energy generation, DNA repair and aging.

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Identifying all of the **proteins** in a human is one thing, but to truly understand a **protein**'s function scientists must discern its shape and structure. In an article in Nature Genetics last October, a group of well-known structural biologists led by Stephen K. Burley of the Rockefeller University called for a "structural genomics initiative" to use quasi-automated x-ray crystallography to study normal and abnormal **proteins**.

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The National Institutes of Health is poised to award \$20 million in grants this year for structural genomics to academic centers. And companies are getting into the game, too: Syrrx in La Jolla, Calif., Structural GenomiX in San Diego, and Chalon Biotech in Toronto are founded on developing so-called high-throughput x-ray crystallographic techniques.

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Added material

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#### FURTHER INFORMATION

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LAURIE GRACE, WITH ASSISTANCE FROM THOMAS NEUBERT New York University AND JOHN YATES Scripps Research Institute; JULIO E. CELIS Danish Center for Human Genome Research. Aarhus (gels); PE CORP. (mass spectrometer)

PAYOFF OF GENOMICS will come in the new fields of transcriptomics and proteomics, which concentrate on determining when and where genes are active and on identifying the **proteins** that those genes encode. JEFF JOHNSON (opposite page); PAUL HARRISON AND MARK GERSTEIN Yale University (**protein** structure); JULIO E. CELIS Danish Center for Human Genome Research, Aarhus (2-D gel)

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#### THE MAJOR PLAYERS

##### CIPHERGEN BIOSYSTEMS

www.ciphergen.com Plans to go public this year

Headquarters: Palo Alto, Calif.

Lead Executive: William E. Rich, president and CEO

Major Clients/Partners: Human Genome Sciences, Parke-Davis, Aventis, SmithKline Beecham

Strategy: Manufacturing and marketing instruments and **chips** for **protein** identification.

Financing This Year: \$28.6 million

Key Challenge: Generating widespread acceptance of mass spectrometry as a common laboratory tool.

Competitive Advantage: A pioneer in using mass spectrometry in **protein** analysis.

##### CURAGEN

www.curagen.com

Stock Symbol: CRGN

Headquarters: New Haven, Conn.

Lead Executive: Jonathan M. Rothberg, president, chairman and CEO

Major Clients/Partners: Pioneer Hi-Bred International, Genentech, Biogen, Glaxo Wellcome

Strategy: Using proteomics to find new drug targets for the company and its partners.

Financing This Year: \$150 million

Key Challenge: Advancing the company's proteomic technologies while developing its own drugs.

Competitive Advantage: Large capacity for mapping the interactions of **proteins**.

##### HYBRIGENICS

www.hybrigenics.com Privately Held

Headquarters: Paris

Lead Executive: Donny Strosberg, CEO

Major Clients/Partners: Institut Pasteur, BioSignal, Lynx Therapeutics

Strategies: Providing cell-wide **protein** interaction maps and drug

target discovery and validation services.

Financing This Year: Not disclosed

Key Challenge: Expanding visibility in the U.S.

Competitive Advantage: Delivers thorough analyses with sophisticated bioinformatics.

#### LARGE SCALE BIOLOGY

www.lsbcb.com Plans to go public this year

Headquarters: Vacaville, Calif.

Lead Executive: Robert L. Erwin, chairman and CEO

Major Clients/Partners: Glaxo Wellcome Procter & Gamble, Novartis, Genentech, Dow

Strategy: Providing **protein** -focused technologies and information to the life sciences industry.

Financing This Year: Not available

Key Challenge: Integrating and promoting business successfully on the heels of its recent merger and initial public offering.

Competitive Advantage: 15 years' experience in proteomics.

#### OXFORD GLYCOSCIENCES

www.ogs.com

Stock Symbol: OGS (London)

Headquarters: Oxford, England

Lead Executive: Michael Kranda, CEO

Major Clients/Partners: Pfizer, Bayer, Merck, G.D. Searle, Incyte Genomics

Strategy: Using proteomics and the study of sugar molecules attached to **proteins** (glycobiology and glycochemistry) to discover new therapeutics and diagnostics.

Financing This Year: £ 33.2 million (\$49.5 million)

Key Challenge: Seeing clinical validation of its efforts.

Competitive Advantage: Highly automated, large-scale analyses of **protein** levels.

#### MYRIAD GENETICS

www.myriad.com

Stock Symbol: MYGN

Headquarters: Salt Lake City

Lead Executive: Peter D. Meldrum, director, president and CEO

Major Clients/Partners: Bayer, Eli Lilly, Pharmacia, Novartis, Roche, Schering and Schering-Plough

Strategies: Selling genetic tests; providing data on **protein** - **protein** interactions to help clients find drug targets.

Financing This Year: None

Key Challenge: Running a service business along with developing its own drugs and maintaining its molecular diagnostics business.

Competitive Advantage: Established company that is expanding its business with strong partners.

#### AFFYMETRIX

www.affymetrix.com

Stock Symbol: AFFX

Headquarters: Santa Clara, Calif.

Lead Executive: Stephen P.A. Fodor, chairman and CEO

Major Clients/Partners: Has installed more than 250 GeneChip systems worldwide

Strategy: Selling **gene chips** and **chip** scanners for research and diagnostic use.

Financing This Year: \$150 million

Key Challenge: Finding new markets for its **chips** and scanners.

Competitive Advantage: The first company to make a business out of **gene chips**.

PAUL HARRISON AND MARK GERSTEIN Yale University; SOURCES: THE COMPANIES LISTED HERE; JAMES D. MCCAMANT AND KIMBERLY TANNER Medical Technology Stock Letter

eds

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Set      Items  Description
S1       1      (PROTEIN? OR PEPTID?) AND ENZYM? AND MICROARRAY? AND (DOT?
              OR MICRODOT?) AND (SLIDE? OR CHIP?) AND (THREE()DIMENSION? OR
              3()DIMENSION?)
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imension?) and (dot? or microdot? or spot? or pad?) and (slide? or chip?)
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12736    PEPTID?
27253    ENZYM?
137      MICROARRAY?
39357    THREE
10012    DIMENSION?
3290     THREE(W)DIMENSION?
85733    3
10012    DIMENSION?
336      3(W)DIMENSION?
0        ENZYM?(20N)MICROARRAY?(20N) (THREE(W)DIMENSION? OR
3(W)DIMENSION?)
1920     DOT?
7        MICRODOT?
5800     SPOT?
1800     PAD?
1407     SLIDE?
2486     CHIP?
S2       0      (PROTEIN? OR PEPTID?) AND ENZYM? (N20) MICROARRAY?
              (N20)((THREE()DIMENSION?) OR 3()DIMENSION?) AND (DOT? OR
MICRODOT? OR SPOT? OR PAD?) AND (SLIDE? OR CHIP?)

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$1.26     0.524 DialUnits File98
$1.55     1 Type(s) in Format 5
$3.15     1 Type(s) in Format 7
$4.70     2 Types
$5.96     Estimated cost File98
$1.39     TELNET
$7.35     Estimated cost this search
$48.36    Estimated total session cost 29.791 DialUnits

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989      PEPTID?
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31       MICROARRAY?
6510     DOT?
17       MICRODOT?
7661     SLIDE?
13600    CHIP?
134323   THREE
11775    DIMENSION?
2746     THREE(W)DIMENSION?
91265    3
11775    DIMENSION?
184      3(W)DIMENSION?
S1       1      (PROTEIN? OR PEPTID?) AND ENZYM? AND MICROARRAY? AND
              (DOT? OR MICRODOT?) AND (SLIDE? OR CHIP?) AND
              (THREE()DIMENSION? OR 3()DIMENSION?)
11065    PROTEIN?
989      PEPTID?

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3058 ENZYM?  
 31 MICROARRAY?  
 134323 THREE  
 11775 DIMENSION?  
 2746 THREE(W)DIMENSION?  
 91265 3  
 11775 DIMENSION?  
 184 3(W)DIMENSION?  
 0 ENZYM?(20N)MICROARRAY?(20N)(THREE(W)DIMENSION? OR  
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 6510 DOT?  
 17 MICRODOT?  
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 7661 SLIDE?  
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04291493 H.W. WILSON RECORD NUMBER: BRGA00041493 (THIS IS THE FULLTEXT)  
**Beyond the human genome.**  
 Ezzell, Carol.  
 Scientific American v. 283 no1 (July 2000) p. 64-9  
 LANGUAGE: English  
 WORD COUNT: 3500

TEXT:

Genes are all the rage right now, but in a sense, at this very moment, they are also becoming passe. Now that all the 100,000 or so genes that make up the human genome have been deciphered, a new industry is emerging to capitalize on when and where those genes are active and on identifying and determining the properties of the **proteins** the genes encode. The enterprise, which has so far attracted hundreds of millions of dollars in venture capital and other financing, can be lumped under the newly coined term "proteomics."

"The biggest issue for genomics today is no longer genes," asserts William A. Haseltine, chairman and chief executive officer of Human Genome Sciences in Rockville, Md. "What's interesting is what you do with those genes."

"We have to move on to understand the other elements of the biological process and couple all this information together," agrees Peter Barrett, chief business officer of Celera Genomics, also in Rockville, the company that raced the publicly funded Human Genome Project to sequence the human genome see "The Human Genome Business Today," on page 50. "People took it for granted that the human genome would be done this year. Now it's 'What do we do next?'"

What's next, for the most part, are messenger RNAs (mRNAs) and **proteins**. If DNA is the set of master blueprints a cell uses to construct **proteins**, then mRNA is like the copy of part of the blueprint that a contractor takes to the building site every day. DNA remains in the nucleus of a cell; mRNAs transcribed from active genes leave the nucleus to give the orders for making **proteins**.

Although every cell in the body contains all of the DNA code for making and maintaining a human being, many of those genes are never "turned on," or copied into mRNA, once embryonic development is complete. Various other genes are turned on or off at different times--or not at all--according to the tissue they are in and their role in the body. A pancreatic beta cell, for instance, is generally full of the mRNA instructions for making insulin, whereas a nerve cell in the brain usually isn't.

Scientists used to think that one gene equals one mRNA equals one **protein**, but the reality is much more complicated. They now know that one gene can be read out in portions that are spliced and diced to generate a

variety of mRNAs and that subsequent processing of the newly made **proteins** that those transcripts encode can alter their function. The DNA sequence of the human genome therefore tells only a small fraction of the story about what a specific cell is doing. Instead researchers must also pay attention to the transcriptome--the body of mRNAs being produced by a cell at any given time--and the proteome, all the **proteins** being made according to the instructions in those mRNAs.

#### CASHING IN ON CHIPS

One of the technologies for studying the human transcriptome is the GeneChip system developed by Affymetrix in Santa Clara, Calif. The system is based on thumbnail-size glass **chips** called **microarrays** that are coated with a thin layer of so-called cDNAs, which represent all the mRNAs made by a particular type of cell. (The abbreviation cDNA stands for complementary DNA; it is essentially mRNA artificially translated back into DNA, but without the noncoding sequence gaps, or introns, found in the original genomic DNA.)

To use the system, scientists isolate mRNA from their cellular sample, tag it with a chemical marker and pour it over the **chip**. By observing where the sample mRNA matches and binds to the cDNA on the **chip**, they can identify the mRNA sequences in their sample. Earlier this year Affymetrix launched two new sets of **chips** for analyzing human cell samples. One allows researchers to identify more than 60,000 different human mRNAs; the other can screen cells for roughly 1,700 human mRNAs related to cancer.

The National Cancer Institute in Bethesda, Md., has been examining the mRNAs produced by various types of cancer cells for more than two years now, in a project called the Human Tumor Gene Index. The index is a partnership between government and academic laboratories as well as a group of drug companies that includes BristolMyers Squibb, Genentech, Glaxo Wellcome and Merck. So far they have identified more than 50,000 genes that are active in one or more cancers. For instance, the index has found that 5,692 genes are active in breast cancer cells, including 277 that are not active in other tissues. Compounds that home in on the **proteins** produced by those 277 genes might serve as good cancer drugs with fewer side effects than current chemotherapies. The National Cancer Institute has also recently begun a multi-million-dollar Tissue Proteomics Initiative in conjunction with the U.S. Food and Drug Administration to identify **proteins** involved in cancer.

At bottom, mRNA studies are just a means to better understand the **proteins** in a cell's production line--after all, the **proteins** are the drug targets. And with researchers expecting that the 100,000 or so human genes will turn out to produce more than a million **proteins**, that's a lot of targets. Jean-Francois Formela of Atlas Venture in Boston estimates that within the next decade the pharmaceutical industry will be faced with evaluating up to 10,000 human **proteins** against which new therapeutics might be directed. That's 25 times the number of drug targets that have been evaluated by all pharmaceutical companies since the dawn of the industry, he says.

Mark J. Levin, CEO of Millennium Pharmaceuticals in Cambridge, Mass., says that large pharmaceutical companies, or "big pharma," need to identify between three and five new drug candidates a year in order to grow 10 to 20 percent a year--the minimum increase shareholders will tolerate. "Right now the major pharma companies are only delivering a half to one-and-a-half entities a year," Levin explains. "Their productivity will not sustain their ability to continue to develop and create shareholder value." Millennium has a relationship with Bayer to deliver 225 pretested "druggable" targets within a few years.

"Protein expression is now capturing the imagination of scientists," comments Randall W. Scott, chief scientific officer of Incyte Genomics in Palo Alto, Calif. "It's being able to look not just at a gene and how it's expressed, but at the forms of the **protein**."

#### PROTEIN MACHINES

Scientists at the DNA-sequencing juggernaut Celera are among those getting interested in the study of **protein** expression, or proteomics. Celera has been in negotiations with GeneBio, a commercial adjunct of the Swiss Institute for Bioinformatics in Geneva, to launch a company dedicated to deducing the entire human proteome. Last year Denis F. Hochstrasser, one of



the founders of GeneBio, and his colleagues published plans for a molecular scanner that would automate the now tedious process of separating and identifying the thousands of **protein** types in a cell.

The current method for studying **proteins** consists in part of a technique called two-dimensional gel electrophoresis, which separates **proteins** by charge and size. In the technique, researchers squirt a solution of cell contents onto a narrow polymer strip that has a gradient of acidity. When the strip is exposed to an electric current, each **protein** in the mixture settles into a layer according to its charge. Next, the strip is placed along the edge of a flat gel and exposed to electricity again. As the **proteins** migrate through the gel, they separate according to their molecular weight. What results is a smudgy pattern of **dots**, each of which contains a different **protein**.

In academic laboratories, scientists generally use a tool similar to a hole puncher to cut the **protein** spots from 2-D gels for individual identification by another method, mass spectroscopy see box on opposite page. But the companies Large Scale Biology in Vacaville, Calif., and Oxford GlycoSciences (OGS) in Oxford, England, use robots to do it. OGS is under contract with Pfizer to analyze samples of cerebrospinal fluid taken from patients with various stages of Alzheimer's disease.

The machine devised by Hochstrasser and his research group goes one step further than the robots used by Large Scale Biology and OGS. It would automatically extract the **protein** spots from the gels, use **enzymes** to chop the **proteins** into bits, feed the pieces into a laser mass spectrometer and transfer the information to a computer for analysis. The instrument manufacturer PE Corporation, which owns Celera, has already agreed to make the machines.

With or without robotic arms, 2-D gels have their problems. Besides being tricky to make, they don't resolve highly charged or low-mass **proteins** very well. They also do a poor job of resolving **proteins** with hydrophobic regions, such as those that span the cell membrane. This is a major limitation, because membrane-spanning receptors are important drug targets.

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Added material

Carol Ezzell, staff writer

#### FURTHER INFORMATION

THE **CHIPPING** FORECAST. A special supplement to Nature Genetics, Vol. 21, No. 1; January 1999.

PROTEOMICS GEARS UP. K. Garber in the on-line publication Signals ([www.signalsmag.com](http://www.signalsmag.com)); November 2, 1999.

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LAURIE GRACE, WITH ASSISTANCE FROM THOMAS NEUBERT New York University AND JOHN YATES Scripps Research Institute; JULIO E. CELIS Danish Center for Human Genome Research, Aarhus (gels); PE CORP. (mass spectrometer)

PAYOFF OF GENOMICS will come in the new fields of transcriptomics and proteomics, which concentrate on determining when and where genes are active and on identifying the **proteins** that those genes encode. JEFF JOHNSON (opposite page); PAUL HARRISON AND MARK GERSTEIN Yale University (protein structure); JULIO E. CELIS Danish Center for Human Genome Research, Aarhus (2-D gel)

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3 Look for color change, which indicates "prey" **protein** binding to "bait"

#### THE MAJOR PLAYERS

##### CIPHERGEN BIOSYSTEMS

[www.ciphergen.com](http://www.ciphergen.com) Plans to go public this year

Headquarters: Palo Alto, Calif.

Lead Executive: William E. Rich, president and CEO

Major Clients/Partners: Human Genome Sciences, Parke-Davis, Aventis, SmithKline Beecham

Strategy: Manufacturing and marketing instruments and chips for **protein** identification.

Financing This Year: \$28.6 million

Key Challenge: Generating widespread acceptance of mass spectrometry as a common laboratory tool.

Competitive Advantage: A pioneer in using mass spectrometry in **protein** analysis.

##### CURAGEN

[www.curagen.com](http://www.curagen.com)

Stock Symbol: CRGN

Headquarters: New Haven, Conn.

Lead Executive: Jonathan M. Rothberg, president, chairman and CEO

Major Clients/Partners: Pioneer Hi-Bred International, Genentech, Biogen, Glaxo Wellcome

Strategy: Using proteomics to find new drug targets for the company and its partners.

Financing This Year: \$150 million

Key Challenge: Advancing the company's proteomic technologies while developing its own drugs.

Competitive Advantage: Large capacity for mapping the interactions of **proteins**.

##### HYBRIGENICS

[www.hybrigenics.com](http://www.hybrigenics.com) Privately Held

Headquarters: Paris  
Lead Executive: Donny Strosberg, CEO  
Major Clients/Partners: Institut Pasteur, BioSignal, Lynx Therapeutics  
Strategies: Providing cell-wide **protein** interaction maps and drug target discovery and validation services.  
Financing This Year: Not disclosed  
Key Challenge: Expanding visibility in the U.S.  
Competitive Advantage: Delivers thorough analyses with sophisticated bioinformatics.

#### LARGE SCALE BIOLOGY

www.lsbcb.com Plans to go public this year

Headquarters: Vacaville, Calif.  
Lead Executive: Robert L. Erwin, chairman and CEO  
Major Clients/Partners: Glaxo Wellcome Procter & Gamble, Novartis, Genentech, Dow  
Strategy: Providing **protein** -focused technologies and information to the life sciences industry.  
Financing This Year: Not available  
Key Challenge: Integrating and promoting business successfully on the heels of its recent merger and initial public offering.  
Competitive Advantage: 15 years' experience in proteomics.

#### OXFORD GLYCOSCIENCES

www.ogs.com

Stock Symbol: OGS (London)  
Headquarters: Oxford, England  
Lead Executive: Michael Kranda, CEO  
Major Clients/Partners: Pfizer, Bayer, Merck, G.D. Searle, Incyte Genomics  
Strategy: Using proteomics and the study of sugar molecules attached to **proteins** (glycobiology and glycochemistry) to discover new therapeutics and diagnostics.  
Financing This Year: £ 33.2 million (\$49.5 million)  
Key Challenge: Seeing clinical validation of its efforts.  
Competitive Advantage: Highly automated, large-scale analyses of **protein** levels.

#### MYRIAD GENETICS

www.myriad.com

Stock Symbol: MYGN  
Headquarters: Salt Lake City  
Lead Executive: Peter D. Meldrum, director, president and CEO  
Major Clients/Partners: Bayer, Eli Lilly, Pharmacia, Novartis, Roche, Schering and Schering-Plough  
Strategies: Selling genetic tests; providing data on **protein** - **protein** interactions to help clients find drug targets.  
Financing This Year: None  
Key Challenge: Running a service business along with developing its own drugs and maintaining its molecular diagnostics business.  
Competitive Advantage: Established company that is expanding its business with strong partners.

#### AFFYMETRIX

www.affymetrix.com

Stock Symbol: AFFX  
Headquarters: Santa Clara, Calif.  
Lead Executive: Stephen P.A. Fodor, chairman and CEO  
Major Clients/Partners: Has installed more than 250 GeneChip systems worldwide  
Strategy: Selling gene **chips** and **chip** scanners for research and diagnostic use.  
Financing This Year: \$150 million  
Key Challenge: Finding new markets for its **chips** and scanners.

Competitive Advantage: The first company to make a business out of gene chips .

PAUL HARRISON AND MARK GERSTEIN Yale University; SOURCES: THE COMPANIES LISTED HERE; JAMES D. MCCAMANT AND KIMBERLY TANNER Medical Technology Stock Letter

?b 411

30apr01 14:49:07 User026066 Session D6422.4  
Sub account: 3776-010140-LAUNCHCYTE-BEJ  
\$0.84 0.349 DialUnits File141  
\$2.85 1 Type(s) in Format 7  
\$2.85 1 Types  
\$3.69 Estimated cost File141  
\$0.60 TELNET  
\$4.29 Estimated cost this search  
\$52.65 Estimated total session cost 30.140 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)

(c) 2001 The Dialog Corporation plc

\*\*\* DIALINDEX search results display in an abbreviated \*\*\*  
\*\*\* format unless you enter the SET DETAIL ON command. \*\*\*  
?sf all

You have 576 files in your file list.

(To see banners, use SHOW FILES command)

?exs td088

>>>"EXS" command not valid in DIALINDEX.

?s (protein? or peptid?) and enzyme (n20) microarray? (n20) ((three()dimension?) or 3()  
dimension?) and (dot? or microdot? or spot? or pad?) and (slide? or chip?)

Your SELECT statement is:

s (protein? or peptid?) and enzyme (n20) microarray? (n20)  
((three()dimension?) or 3()dimension?) and (dot? or microdot? or spot? or  
pad?) and (slide? or chip?)

Items	File
Examined 50 files	
Examined 100 files	
Examined 150 files	
Examined 200 files	
Examined 250 files	
Examined 300 files	
Examined 350 files	
Examined 400 files	
Examined 450 files	
Examined 500 files	
Examined 550 files	

No files have one or more items; file list includes 576 files.  
One or more terms were invalid in 2 files.

?logoff hold

30apr01 14:59:50 User026066 Session D6422.5  
Sub account: 3776-010140-LAUNCHCYTE-BEJ  
\$16.82 13.453 DialUnits File411  
\$16.82 Estimated cost File411  
\$2.20 TELNET  
\$19.02 Estimated cost this search  
\$71.67 Estimated total session cost 43.594 DialUnits

### Status: Signed Off. (42 minutes)

### Status: Path 1 of [Dialog]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID dialog.com)  
Trying 3106...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 00.12.12D

Last logoff: 30apr01 15:30:22

Logon file001 30apr01 16:00:02

NOTICE set ON to \$20.00

You will be prompted to confirm each TYPE or PRINT request where  
format charges exceeds \$20.00

Please enter SUBACCOUNT name/number:

?3776-010140-BEJ-launchcyte

Is 3776-010140-BEJ-LAUNCHCYTE the SUBACCOUNT you want to use? (Y/N)

?y

Subaccount is set to 3776-010140-BEJ-LAUNCHCYTE

\*\*\*

\*\*\*

File 1:ERIC 1966-2001/Apr 17

(c) format only 2001 The Dialog Corporation

Set Items Description

-----

Terminal set to DLINK

?b 411

30apr01 16:00:25 User026066 Session D6423.1

Sub account: 3776-010140-BEJ-LAUNCHCYTE

\$0.19 0.056 DialUnits File1

\$0.19 Estimated cost File1

\$0.07 TELNET

\$0.26 Estimated cost this search

\$0.26 Estimated total session cost 0.056 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)

(c) 2001 The Dialog Corporation plc

\*\*\* DIALINDEX search results display in an abbreviated \*\*\*  
\*\*\* format unless you enter the SET DETAIL ON command. \*\*\*

?sf all

You have 576 files in your file list.

(To see banners, use SHOW FILES command)

?s (protein?/ti or peptide?/ti) and microarray/ti

Your SELECT statement is:

s (protein?/ti or peptide?/ti) and microarray/ti

Items File

-----

1	2: INSPEC_1969-2001/Apr W5
15	5: Biosis Previews(R)_1969-2001/Apr W4
1	8: Ei Compendex(R)_1970-2001/Apr W1
1	16: Gale Group PROMT(R)_1990-2001/Apr 27
2	20: World Reporter_1997-2001/Apr 30
13	34: SciSearch(R) Cited Ref Sci_1990-2001/Apr W5

Examined 50 files

5 71: ELSEVIER BIOBASE\_1994-2001/Apr W4  
6 73: EMBASE\_1974-2001/Apr W4  
2 76: Life Sciences Collection\_1982-2001/Feb  
2 88: Gale Group Business A.R.T.S.\_1976-2001/Apr 30  
1 94: JICST-EPlus\_1985-2001/Apr W2  
2 98: General Sci Abs/Full-Text\_1984-2001/Mar  
1 99: Wilson Appl. Sci & Tech Abs\_1983-2001/Mar

>>>Term "TI" is not a valid suffix in file 100 and is ignored

1 100: Market Guide Company Financials\_2001/Apr 30

>>>Term "TI" is not defined in file 107 and is ignored

1 107: Adis R&D Insight\_1986-2001/Apr W3

1 111: TGG Natl.Newspaper Index(SM)\_1979-2001/Apr 25

>>>Term "TI" is not defined in file 115 and is ignored

4 115: Research Centers & Services\_1994-2000/Nov

Examined 100 files

>>>Term "TI" is not defined in file 133 and is ignored

1 133: S&P's Corp.Descrip.+News\_2001/Apr 30

1 143: Biol. & Agric. Index\_1983-2001/Mar

6 144: Pascal\_1973-2001/Apr W5

1 148: Gale Group Trade & Industry DB\_1976-2001/Apr 27

1 149: TGG Health&Wellness DB(SM)\_1976-2001/Apr W4

1 151: HealthSTAR\_1975-2000/Dec

5 155: MEDLINE(R)\_1966-2001/May W3

2 159: Cancerlit\_1975-2001/Mar

1 165: EventLine(TM)\_1990-2001/Mar

1 172: EMBASE Alert\_2001/Apr W4

1 174: Pharm-line(R)\_1978-2001/Apr W4

Examined 150 files

Examined 200 files

>>>Term "TI" is not defined in file 286 and is ignored

12 286: Biocommerce Abs.& Dir.\_1981-2001/Apr B1

1 342: Derwent Patents Citation Indx\_1978-01/200120

Examined 250 files

4 351: Derwent WPI\_1963-2001/UD,UM &UP=200123

8 357: Derwent Biotechnology Abs\_1982-2001/May B1

1 377: Derwent Drug File\_1983-2001/May W2

5 399: CA SEARCH(R)\_1967-2001/UD=13418

5 420: UnCover\_1988-2001/Apr 30

1 431: MediConf: Medical Con. & Events\_1998-2001/Apr B2

Examined 300 files

145 440: Current Contents Search(R)\_1990-2001/May W1

>>>Term "TI" is not defined in file 453 and is ignored

3 453: Drugs of the Future\_1990-2001/Mar

2 484: Periodical Abstracts Plustext\_1986-2001/Apr W4

>>>Term "TI" is not defined in file 501 and is ignored

2 501: Extel Intl News Cards\_1995-2001/Apr W4

>>>Term "TI" is not defined in file 505 and is ignored

1 505: Asian Co. Profiles\_2001/Jan

Examined 350 files

>>>Term "TI" is not defined in file 519 and is ignored

1 519: D&B-Duns Finan.Records Plus(TM)\_2000/Nov

Examined 400 files

2 613: PR Newswire\_1999-2001/Apr 30

1 621: Gale Group New Prod.Annou.(R)\_1985-2001/Apr 27

Examined 450 files

1 649: Gale Group Newswire ASAP(TM)\_2001/Apr 25

3 660: Federal News Service\_1991-2001/Mar 08

Examined 500 files

Examined 550 files

46 files have one or more items; file list includes 576 files.  
One or more terms were invalid in 132 files.

?

PLEASE ENTER A COMMAND OR BE LOGGED OFF IN 5 MINUTES

?b 2,5,8,16

30Apr01 16:11:45 User026066 Session D6423.2

Sub account: 3776-010140-BEU-LAUNCHCYTE

\$7.31 5.850 DialUnits File411  
 \$7.31 Estimated cost File411  
 \$2.40 TELNET  
 \$9.71 Estimated cost this search  
 \$9.97 Estimated total session cost 5.906 DialUnits

SYSTEM:OS - DIALOG OneSearch

File 2:INSPEC 1969-2001/Apr W5  
 (c) 2001 Institution of Electrical Engineers  
 File 5:Biosis Previews(R) 1969-2001/Apr W4  
 (c) 2001 BIOSIS  
 File 8:EI Compendex(R) 1970-2001/Apr W1  
 (c) 2001 Engineering Info. Inc.  
 File 16:Gale Group PROMT(R) 1990-2001/Apr 27  
 (c) 2001 The Gale Group

Set Items Description

7b 2,5,8,16,20,34,71,73,76,88,94,98,99,100,107,111,115,133,143,144,148,149,151,155,159,  
 165,172,174,286,342,351,357,377,399,420,431,440,453,484,501,505,519,613,621,649,660  
 30apr01 16:13:48 User026066 Session D6423.3  
 Sub account: 3776-010140-BEJ-LAUNCHCYTE  
 \$0.09 0.015 DialUnits File2  
 \$0.09 Estimated cost File2  
 \$0.09 0.015 DialUnits File5  
 \$0.09 Estimated cost File5  
 \$0.10 0.015 DialUnits File8  
 \$0.10 Estimated cost File8  
 \$0.08 0.015 DialUnits File16  
 \$0.08 Estimated cost File16  
 OneSearch, 4 files, 0.061 DialUnits FileOS  
 \$0.60 TELNET  
 \$0.96 Estimated cost this search  
 \$10.93 Estimated total session cost 5.967 DialUnits

SYSTEM:OS - DIALOG OneSearch

File 2:INSPEC 1969-2001/Apr W5  
 (c) 2001 Institution of Electrical Engineers  
 File 5:Biosis Previews(R) 1969-2001/Apr W4  
 (c) 2001 BIOSIS  
 File 8:EI Compendex(R) 1970-2001/Apr W1  
 (c) 2001 Engineering Info. Inc.  
 File 16:Gale Group PROMT(R) 1990-2001/Apr 27  
 (c) 2001 The Gale Group  
 File 20:World Reporter 1997-2001/Apr 30  
 (c) 2001 The Dialog Corporation  
 File 34:SciSearch(R) Cited Ref Sci 1990-2001/Apr W5  
 (c) 2001 Inst for Sci Info  
 File 71:ELSEVIER BIOBASE 1994-2001/Apr W4  
 (c) 2001 Elsevier Science B.V.  
 File 73:EMBASE 1974-2001/Apr W4  
 (c) 2001 Elsevier Science B.V.

\*File 73: For information about Explode feature please see Help News73.

File 76:Life Sciences Collection 1982-2001/Feb  
 (c) 2001 Cambridge Sci Abs  
 File 88:Gale Group Business A.R.T.S. 1976-2001/Apr 30  
 (c) 2001 The Gale Group  
 File 94:JICST-EPlus 1985-2001/Apr W2  
 (c) 2001 Japan Science and Tech Corp(JST)

\*File 94: There is no data missing. UDs have been adjusted to reflect the current months data. See Help News94 for details.

File 98:General Sci Abs/Full-Text 1984-2001/Mar  
 (c) 2001 The HW Wilson Co.  
 File 99:Wilson Appl. Sci & Tech Abs 1983-2001/Mar  
 (c) 2001 The HW Wilson Co.  
 File 100:Market Guide Company Financials 2001/Apr 30



(c) 2001 Market Guide  
File 107:Adis R&D Insight 1986-2001/Apr W3  
(c) 2001 Adis International Ltd.  
File 111:TGG Natl.Newspaper Index(SM) 1979-2001/Apr 25  
(c) 2001 The Gale Group  
File 115:Research Centers & Services 1994-2000/Nov  
(c) 2000 Gale Research Inc.  
File 133:S&P's Corp.Descrip.+News 2001/Apr 30  
(c) 2001 McGraw-Hill Co. Inc  
File 143:Biol. & Agric. Index 1983-2001/Mar  
(c) 2001 The HW Wilson Co  
File 144:Pascal 1973-2001/Apr W5  
(c) 2001 INIST/CNRS  
File 148:Gale Group Trade & Industry DB 1976-2001/Apr 27  
(c)2001 The Gale Group  
File 149:TGG Health&Wellness DB(SM) 1976-2001/Apr W4  
(c) 2001 The Gale Group  
File 151:HealthSTAR 1975-2000/Dec  
(c) format only 2000 The Dialog Corporation  
**\*File 151: Final updates for this file have been loaded and the file is now closed. Please see Help News151 for changes to the file.**  
File 155:MEDLINE(R) 1966-2001/May W3  
(c) format only 2000 Dialog Corporation  
**\*File 155: Medline has now updated. For further information see Help News155.**  
File 159:Cancerlit 1975-2001/Mar  
(c) format only 2001 Dialog Corporation  
File 165:EventLine(TM) 1990-2001/Mar  
(c) 2001 Elsevier Science B.V.  
**\*File 165: Updates are currently restricted to Medical and Biotechnical events only.**  
File 172:EMBASE Alert 2001/Apr W4  
(c) 2001 Elsevier Science B.V.  
File 174:Pharm-line(R) 1978-2001/Apr W4  
(c) CROWN COPYRIGHT 2001  
**\*File 174: UDs have been readjusted to reflect the current months data. There is no data missing.**  
File 286:Biocommerce Abs.& Dir. 1981-2001/Apr B1  
(c) 2001 BioCommerce Data Ltd.  
File 342:Derwent Patents Citation Indx 1978-01/200120  
(c) 2001 Derwent Info Ltd  
**\*File 342: Price changes as of 1/1/01. Please see HELP RATES 342.**  
File 351:Derwent WPI 1963-2001/UD,UM &UP=200123  
(c) 2001 Derwent Info Ltd  
**\*File 351: Price changes as of 1/1/01. Please see HELP RATES 351. 72 Updates in 2001. Please see HELP NEWS 351 for details.**  
File 357:Derwent Biotechnology Abs 1982-2001/May B1  
(c) 2001 Derwent Publ Ltd  
**\*File 357: Price changes as of 1/1/01. Please see HELP RATES 357.**  
File 377:Derwent Drug File 1983-2001/May W2  
(c) 2001 Derwent Info Ltd.  
File 399:CA SEARCH(R) 1967-2001/UD=13418  
(c) 2001 AMERICAN CHEMICAL SOCIETY  
**\*File 399: Use is subject to the terms of your user/customer agreement. RANK charge added; see HELP RATES 399.**  
File 420:UnCover 1988-2001/Apr 30  
(c) 2001 The UnCover Company  
**\*File 420: Please check rates (enter r from the main menu) for important information about patent collections and availability.**  
File 431:MediConf: Medical Con. & Events 1998-2001/Apr B2  
(c) 2001 Dr. R. Steck  
**\*File 431: There is no data missing. UDs have been adjusted to reflect the current months data.**  
File 440:Current Contents Search(R) 1990-2001/May W1  
(c) 2001 Inst for Sci Info  
File 453:Drugs of the Future 1990-2001/Mar  
(c) 2001 Prous Science  
File 484:Periodical Abstracts Plustext 1986-2001/Apr W4  
(c) 2001 Bell & Howell

File 501:Extel Intl News Cards 1995-2001/Apr W4  
 (c) 2001 Extel Financial Inc  
 File 505:Asian Co. Profiles 2001/Jan  
 (c) 2001 FBR Bus Info Svcs  
 \*File 505: Records with financial data are not  
 formatting correctly; see HELP NEWS 505.  
 File 519:D&B-Duns Finan.Records Plus(TM) 2000/Nov  
 (c) 2001 Dun & Bradstreet  
 \*File 519: Enter REPORT Sn/BIR, or REPORT Sn/SER, or REPORT Sn/PAR  
 to receive special reports directly from D&B.  
 File 613:PR Newswire 1999-2001/Apr 30  
 (c) 2001 PR Newswire Association Inc  
 \*File 613: File 613 now contains data from 5/99 forward.  
 Archive data (1987-4/99) is available in File 813.  
 File 621:Gale Group New Prod.Annou.(R) 1985-2001/Apr 27  
 (c) 2001 The Gale Group  
 File 649:Gale Group Newswire ASAP(TM) 2001/Apr 25  
 (c) 2001 The Gale Group  
 File 660:Federal News Service 1991-2001/Mar 08  
 (c) 2001 Federal News Service  
 \*File 660: This file is temporarily not updating.

Set Items Description

-----  
 ?s (protein?/ti or peptid?/ti) and microarray?/ti  
 Processing  
 Processed 10 of 46 files ...  
 >>>Term "TI" is not a valid suffix in one or more files  
 Processing  
 Processed 20 of 46 files ...  
 Processing  
 Processed 30 of 46 files ...  
 Completed processing all files  
 3171061 PROTEIN?/TI  
 748057 PEPTID?/TI  
 5607 MICROARRAY?/TI  
 S1 513 (PROTEIN?/TI OR PEPTID?/TI) AND MICROARRAY?/TI

?rd  
 >>>Duplicate detection is not supported for File 100.  
 >>>Duplicate detection is not supported for File 107.  
 >>>Duplicate detection is not supported for File 115.  
 >>>Duplicate detection is not supported for File 133.  
 >>>Duplicate detection is not supported for File 165.  
 >>>Duplicate detection is not supported for File 174.  
 >>>Duplicate detection is not supported for File 286.  
 >>>Duplicate detection is not supported for File 342.  
 >>>Duplicate detection is not supported for File 351.  
 >>>Duplicate detection is not supported for File 431.  
 >>>Duplicate detection is not supported for File 453.  
 >>>Duplicate detection is not supported for File 501.  
 >>>Duplicate detection is not supported for File 505.  
 >>>Duplicate detection is not supported for File 660.  
 >>>Records from unsupported files will be retained in the RD set.  
 ...examined 50 records (50)  
 ...examined 50 records (100)  
 ...examined 50 records (150)  
 ...examined 50 records (200)  
 >>>Record 440:12608814 ignored; incomplete bibliographic data, not retained  
 in RD set  
 >>>Record 440:12604523 ignored; incomplete bibliographic data, not retained  
 in RD set  
 >>>Record 440:12603589 ignored; incomplete bibliographic data, not retained  
 in RD set  
 >>>Record 440:12598931 ignored; incomplete bibliographic data, not retained  
 in RD set  
 >>>Record 440:12598733 ignored; incomplete bibliographic data, not retained  
 in RD set  
 >>>Record 440:12590270 ignored; incomplete bibliographic data, not retained

in RD set  
>>>Record 440:12581700 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12577692 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12575518 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12570693 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12567272 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12566990 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12565144 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12564034 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12561732 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12557898 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12545521 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12537476 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12535845 ignored; incomplete bibliographic data, not retained  
in RD set  
...examined 50 records (250)  
>>>Record 440:12528591 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12528496 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12520748 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12519368 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12517947 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12507378 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12505656 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12489778 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12489617 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12483108 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12482822 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12480260 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12479086 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12477804 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12459226 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12456861 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12441800 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12438611 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12438254 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12419808 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12417860 ignored; incomplete bibliographic data, not retained

in RD set  
>>>Record 440:12417207 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12415928 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12407840 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12407356 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12402545 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12401077 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12399797 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12396974 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12393513 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12366175 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12364113 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12360790 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12357824 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12350031 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12346778 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12345429 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12331578 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12306558 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12293855 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12280833 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12280317 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12278916 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12278743 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12273462 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12269412 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12266383 ignored; incomplete bibliographic data, not retained  
in RD set  
...examined 50 records (300)  
>>>Record 440:12264621 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12247266 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12242836 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12240459 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12237460 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12235525 ignored; incomplete bibliographic data, not retained  
in RD set  
>>>Record 440:12231881 ignored; incomplete bibliographic data, not retained  
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2/5/1 (Item 1 from file: 2)

DIALOG(R)File 2:INSPEC

(c) 2001 Institution of Electrical Engineers. All rts. reserv.

6810039 INSPEC Abstract Number: A2001-04-8715-008

Title: Preparation and characterization of self-assembled double-stranded DNA (dsDNA) microarrays for protein:dsDNA screening using atomic force microscopy

Author(s): O'Brien, J.; Stickney, J.T.; Porter, M.D.

Author Affiliation: Dept. of Chem., Iowa State Univ., Ames, IA, USA

Journal: Langmuir vol.16, no.24 p.9559-67

Publisher: American Chem. Soc.

Publication Date: 28 Nov. 2000 Country of Publication: USA

CODEN: LANGD5 ISSN: 0743-7463

SICI: 0743-7463(20001128)16:24L.9559:PCSA;1-V

Material Identity Number: K859-2000-025

U.S. Copyright Clearance Center Code: 0743-7463/2000/\$19.00

Language: English Document Type: Journal Paper (JP)

Treatment: Experimental (X)

Abstract: We report details on the development of a self-assembled, double-stranded DNA (dsDNA) microarray fabrication strategy suitable for protein:dsDNA screening using the atomic force microscope (AFM). Using disulfide-modified dsDNA (26-mer) synthesized to contain the recognition sequence for EcoRI, we have created micron-sized mixed monolayer surfaces where both the spatial orientation and packing density of the immobilized oligonucleotides, two critical parameters for screening protein:dsDNA interactions, are controlled. Before exposure to EcoRI, the topography of microarrays that were composed of 26-mers containing the recognition sequence for EcoRI was 8.8 nm+or-1.5 nm (n=5), a value consistent with that predicted by X-ray diffraction studies. After enzyme digestion, the topography of the microarray decreased to 4.3 nm+or-0.8 nm (n=14), a value consistent with predictions based on the position of the recognition sequence within the oligonucleotides. In contrast, the topography of microarrays that were composed of 26-mers that did not contain the recognition sequence for EcoRI remained essentially the same before (8.9 nm+or-1.5 nm (n=5)) and after (8.3 nm+or-1.4 nm (n=5)) exposure to EcoRI. Furthermore, because the dsDNA were synthesized to include a fluorescein moiety above the recognition sequence, the loss of fluorescence after exposure to EcoRI was also used to detect enzymatic cleavage. We believe that this technology holds promise as a tool for the rapid and facile screening of multiple protein interactions using massively parallel dsDNA microarrays. (31 Refs)

Subfile: A

Descriptors: arrays; atomic force microscopy; DNA; fluorescence; molecular biophysics; pattern recognition; proteins; self-assembly; X-ray diffraction

Identifiers: preparation; self-assembled double-stranded DNA microarrays; protein:dsDNA screening; atomic force microscopy; self-assembled double-stranded DNA; fabrication strategy; AFM; disulfide-modified dsDNA; recognition sequence; micron-sized mixed monolayer surfaces; spatial orientation; packing density; topography; X-ray diffraction; enzyme digestion; oligonucleotides; fluorescein moiety; fluorescence; enzymatic cleavage; multiple protein interactions; massively parallel dsDNA microarrays

Class Codes: A8715H (Biomolecular dynamics, molecular probes, molecular pattern recognition); A8715B (Biomolecular structure, configuration, conformation, and active sites); A8715K (Biomolecular interactions, charge transfer complexes); A8715M (Interactions with radiations at the biomolecular level)

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?s s2 not DNA/ti

>>>Term "TI" is not a valid suffix in one or more files

120 S2

1266447 DNA/TI

S3

80 S2 NOT DNA/TI

?t 3/5/1

3/5/1 (Item 1 from file: 2)

DIALOG(R)File 2:INSPEC

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6094209 INSPEC Abstract Number: A9901-8715-014

Title: Direct protein microarray fabrication using a hydrogel "stamper"

Author(s): Martin, B.D.; Gaber, B.P.; Patterson, C.H.; Turner, D.C.

Author Affiliation: Naval Res. Lab., Washington, DC, USA

Journal: Langmuir vol.14, no.15 p.3971-5

Publisher: American Chem. Soc,

Publication Date: 21 July 1998 Country of Publication: USA

CODEN: LANGD5 ISSN: 0743-7463

SICI: 0743-7463(19980721)14:15L:3971:DPMF;1-S

Material Identity Number: K859-98015

U.S. Copyright Clearance Center Code: 0743-7463/98/\$15.00

Document Number: S0743-7463(97)01331-0

Language: English Document Type: Journal Paper (JP)

Treatment: Experimental (X)

Abstract: Micropatterned arrays of active proteins are vital to the next generation of high-throughput multiplexed biosensors and advanced medical diagnostics. We have developed a simple method for fabricating antibody arrays using a micromolded hydrogel "stamper" and an aminosilylated receiving surface. The stamping procedure permits direct protein deposition and micropatterning while avoiding cross-contamination of separate patterned regions. Three different antibodies were stamped in adjacent arrays of 50-80  $\mu$ m circular areas with retention of activity. /sup 125/I labeling and atomic force microscopy studies showed that the stamper deposited protein as a submonolayer. The fluorescent signal-to-background ratio of labeled bound antigen was greater than 25:1. (30 Refs)

Subfile: A

Descriptors: atomic force microscopy; biosensors; gels; proteins

Identifiers: direct protein microarray fabrication; hydrogel stamper; micropatterned arrays; active proteins; high-throughput multiplexed biosensors; advanced medical diagnostics; antibody arrays; micromolded hydrogel; aminosilylated receiving surface; /sup 125/I labeling; atomic force microscopy; submonolayer; fluorescent signal-to-background ratio; labeled bound antigen; AFM; I

Class Codes: A8715 (Molecular biophysics); A8780B (Biosensors); A8270G (Gels and sols)

Chemical Indexing:

I el (Elements - 1)

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?t 3/5/2-80

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?t 3/4/2-80

Estimated cost of output requested is: \$248.05

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3/4/2 (Item 1 from file: 5)

FN- DIALOG(R) File 5: Biosis Previews(R) |

CZ- (c) 2001 BIOSIS. All rts. reserv. |

AZ- 12933528 |

AA- 200100140677 |

TI- Protein arrays and microarrays. |

AU- Zhu Heng(a); Snyder Michael(a) |

CS- (a) Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT, 06520: michael.snyder@yale.edu USA |

JN- Current Opinion in Chemical Biology |

VO- 5 |

IS- 1 |

PG- 40-45 |

DA- February, |

PY- 2001 |

ME- print

SN- 1367-5931|  
 DT- Literature Review|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 RN- 50812-37-8: GLUTATHIONE-S-TRANSFERASE|  
 DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular Biophysics); Methods and Techniques|  
 DE- <CHEMICALS> bovine serum albumin; glutathione-S-transferase; protein arrays--applications; protein microarrays--applications; proteins--analysis, functions; proteomes--analysis|  
 DE- <METHODS> genome sequencing--analytical method; large-scale proteome analysis--analytical method|  
 DE- <MISC.> biotechnology; genomes--analysis; methodology; open reading frames; protein-protein interactions--analysis|  
 CC- 10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
 03502 Genetics and Cytogenetics-General  
 10802 Enzymes-General and Comparative Studies; Coenzymes|

3/4/3 (Item 2 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12925186|  
 AA- 200100132335|  
 TI- Association of p27Kip1 protein expression and formalin-fixation:  
 Optimal fixation times determined using high-density tissue microarray  
 .|  
 AU- De Marzo A M; Mucci N R(a); Cushenberry E(a); Rubin M A(a)|  
 CS- (a)U of Michigan, Ann Arbor, MI USA|  
 JN- Laboratory Investigation|  
 VO- 81|  
 IS- 1|  
 PG- 106A|  
 DA- January,|  
 PY- 2001|  
 ME- print|  
 CT- Annual Meeting of the United States and Canadian Academy of Pathology|  
 LO- Atlanta, Georgia, USA|  
 DA- March 03-09, 2001|  
 SN- 0023-6837|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 DE- <MAJOR CONCEPT> Biochemistry and Molecular Biophysics; Oncology (Human Medicine, Medical Sciences); Urology (Human Medicine, Medical Sciences); Methods and Techniques|  
 DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia|  
 DE- <ORGANISMS> human (Hominidae)--patient|  
 DE- <PARTS,ETC> prostate--reproductive system|  
 DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates|  
 DE- <DISEASES> prostate cancer--neoplastic disease, reproductive system disease/male, urologic disease; prostate carcinoma--neoplastic disease, reproductive system disease/male, urologic disease|  
 DE- <CHEMICALS> cyclin-dependent kinase inhibitor p27-Kip-1--analysis, expression, functions; proteins--analysis, expression, functions|  
 DE- <METHODS> formalin fixation--fixation, histological method, optimal fixation times; high-density tissue microarrays--Immunohistochemical/Immunocytochemical Techniques, analytical method, diagnostic method; immunohistochemistry--Immunohistochemical/Immunocytochemical Techniques, analytical method, diagnostic method|  
 DE- <MISC.> cancer prognostic biomarkers--applications; pathology; tissue sections--analysis; Meeting Abstract|  
 DE- <ALT. INDEX> Prostatic Neoplasms (MeSH); Prostatic Neoplasms (MeSH); Carcinoma (MeSH)|  
 CC- 16504 Reproductive System-Physiology and Biochemistry

00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals  
 10060 Biochemical Studies-General  
 10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
 12502 Pathology, General and Miscellaneous-General  
 12504 Pathology, General and Miscellaneous-Diagnostic  
 15506 Urinary System and External Secretions-Pathology  
 16506 Reproductive System-Pathology  
 24001 Neoplasms and Neoplastic Agents-Diagnostic Methods  
 24004 Neoplasms and Neoplastic Agents-Pathology; Clinical Aspects;  
 Systemic Effects|  
 BC- 86215 Hominidae|

3/4/4 (Item 3 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12917402|  
 AA- 200100124551|  
 TI- Correlation of Human Papillomavirus detection with p53, p27, and Ki-67  
 protein expression in penile carcinoma using tissue microarray  
 technology.|  
 AU- Zhou M(a); Pirog E C; Proverbs-Singh T(a); Mucci N R(a); Kleter B;  
 Ayala G; Cubilla A L; Quint W G V; Rubin M A(a)|  
 CS- (a)Univ. of Michigan, Ann Arbor, MI USA|  
 JN- Laboratory Investigation|  
 VO- 81|  
 IS- 1|  
 PG- 131A|  
 DA- January,|  
 PY- 2001|  
 ME- print|  
 CT- Annual Meeting of the United States and Canadian Academy of Pathology|  
 LO- Atlanta, Georgia, USA|  
 DA- March 03-09, 2001|  
 SN- 0023-6837|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 DE- <MAJOR CONCEPT> Biochemistry and Molecular Biophysics; Infection;  
 Reproductive System (Reproduction); Tumor Biology|  
 DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata,  
 Animalia; Papovaviridae--Animal Viruses, Viruses, Microorganisms|  
 DE- <ORGANISMS> human (Hominidae)--host, male; human papillomavirus  
 (Papovaviridae)--pathogen|  
 DE- <SUPER TAXA> Animal Viruses; Animals; Chordates; Humans; Mammals;  
 Microorganisms; Primates; Vertebrates; Viruses|  
 DE- <DISEASES> human papillomavirus infection--carcinogenic complications,  
 viral disease; penile carcinoma--neoplastic disease, reproductive  
 system disease/male|  
 DE- <CHEMICALS> Ki-67--expression; human papillomavirus DNA; p27--  
 expression; p53--expression|  
 DE- <METHODS> tissue microarray--genetic method|  
 DE- <GEO. NAME> Paraguay (South America, Neotropical region); USA (North  
 America, Nearctic region)|  
 DE- <MISC.> Meeting Abstract|  
 DE- <ALT. INDEX> Papovaviridae Infections (MeSH)|  
 CC- 16504 Reproductive System-Physiology and Biochemistry  
 00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals  
 10060 Biochemical Studies-General  
 10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
 16506 Reproductive System-Pathology  
 24004 Neoplasms and Neoplastic Agents-Pathology; Clinical Aspects;  
 Systemic Effects  
 33506 Virology-Animal Host Viruses  
 36006 Medical and Clinical Microbiology-Virology|  
 BC- 02616 Papovaviridae (1993- )  
 86215 Hominidae|

3/4/5 (Item 4 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|

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AZ- 12891221|

AA- 200100098370|

TI- Altered expression of genes related to G protein signaling in  
schizophrenia revealed by high density cDNA microarrays. |

AU- Middleton F A(a); Mirnics K; Marquez A; Stanwood G D; Volk C L; Lewis D  
A; Levitt P|

CS- (a)University of Pittsburgh School of Medicine, Pittsburgh, PA USA|

JN- Society for Neuroscience Abstracts|

VO- 26|

IS- 1-2|

PG- Abstract No-58111|

PY- 2000|

ME- print|

CT- 30th Annual Meeting of the Society of Neuroscience|

LO- New Orleans, LA, USA|

DA- November 04-09, 2000|

SP- Society for Neuroscience; |

SN- 0190-5295|

RT- Abstract|

LA- English|

SL- English|

AB- It has been suggested that the development of schizophrenia may be related to abnormalities in receptor-G protein coupling and downstream second messenger signaling. To address this possibility in a comprehensive fashion, we used high density cDNA microarrays to compare the expression patterns of several hundred genes and functional cascades related to intracellular signaling pathways in post mortem samples of prefrontal cortex area 9 from six matched pairs of schizophrenic and control subjects. Most genes and cascades related to these processes were not significantly different according to this analysis. However, several candidate genes did show consistent and significant decreases across all subject pairs. Moreover, a number of these genes encode proteins that interact in a common signaling pathway. Importantly, alterations in these transcripts were unrelated to treatment of the disorder with neuroleptic medication, because they were also present in the schizophrenic subject who was not receiving medication at the time of death and they were not present in monkeys treated for one year with neuroleptic medication. We are currently verifying the most robust changes in transcript expression and determining the regional specificity and cellular localization of these changes using in situ hybridization, immunohistochemistry and Western blot analysis. Taken together, these data strongly implicate several genes and at least one second messenger pathway in the pathophysiology of schizophrenia. |

DE- <MAJOR CONCEPT> Genetics; Nervous System (Neural Coordination)|

DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Primates--Mammalia, Vertebrata, Chordata, Animalia|

DE- <ORGANISMS> human (Hominidae)--patient; monkey (Primates)|

DE- <PARTS, ETC> prefrontal cortex--nervous system|

DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Nonhuman Mammals; Nonhuman Primates; Nonhuman Vertebrates; Primates; Vertebrates|

DE- <DISEASES> schizophrenia--behavioral and mental disorders|

DE- <CHEMICALS> G protein--signalling; neuroleptic medications; second messengers|

DE- <METHODS> Western blot--analytical method, detection/labeling techniques, gene mapping; high density cDNA microarray--analytical method; immunohistochemistry--analytical method; in situ hybridization--analytical method|

DE- <MISC.> disease development; gene expression; intracellular pathways; Meeting Abstract|

DE- <ALT. INDEX> Schizophrenia (MeSH)|

CC- 03508 Genetics and Cytogenetics-Human

00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals

03502 Genetics and Cytogenetics-General  
 03506 Genetics and Cytogenetics-Animal  
 07004 Behavioral Biology-Human Behavior  
 10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines  
 10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
 20504 Nervous System-Physiology and Biochemistry  
 21002 Psychiatry-Psychopathology; Psychodynamics and Therapy|  
 BC- 86190 Primates-Unspecified  
 86215 Hominidae|

3/4/6 (Item 5 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12805742|  
 AA- 200100012891|

TI- Amphotericin B-induced differential expression of genes encoding immunomodulatory proteins in human monocytic cells detected by cDNA microarray. |

AU- Rogers P D(a); Pearson M M(a); Chapman S W(a); Cleary J D(a)|  
 CS- (a)Univ. of Mississippi Med. Ctr., Jackson, MS USA|  
 JN- Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy|  
 VO- 40|  
 PG- 393|  
 PY- 2000|  
 ME- print|  
 CT- 40th Interscience Conference on Antimicrobial Agents and Chemotherapy|  
 LO- Toronto, Ontario, Canada|  
 DA- September 17-20, 2000|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 RN- 1397-89-3: AMPHOTERICIN B|  
 DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular Biophysics); Blood and Lymphatics (Transport and Circulation)|  
 DE- <PARTS,ETC> monocytic cell--blood and lymphatics|  
 DE- <CHEMICALS> amphotericin B--antifungal-drug; complement; cytokine; cytokine receptor; immunomodulatory proteins|  
 DE- <METHODS> cDNA Microarray--analytical method|  
 DE- <MISC.> cell adhesion; gene expression; Meeting Abstract|  
 CC- 03502 Genetics and Cytogenetics-General  
 00520 General Biology-Symposia, Transactions and Proceedings of Conferences, Congresses, Review Annuals  
 02506 Cytology and Cytochemistry-Animal  
 10060 Biochemical Studies-General  
 10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
 12512 Pathology, General and Miscellaneous-Therapy (1971- )  
 15002 Blood, Blood-Forming Organs and Body Fluids-Blood and Lymph Studies  
 15004 Blood, Blood-Forming Organs and Body Fluids-Blood Cell Studies  
 17002 Endocrine System-General  
 38508 Chemotherapy-Antifungal Agents|

3/4/7 (Item 6 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12771320|  
 AA- 200000524943|

TI- Comparative microarray analysis of gene expression during apoptosis-induction by growth factor deprivation or protein kinase C inhibition. |

AU- Brachat Arndt; Pierrat Benoit; Brungger Adrian; Heim Jutta(a)|  
 CS- (a)Novartis Pharma AG, K-125.13-16, CH-4002, Basel Switzerland|  
 JN- Oncogene|  
 VO- 19|  
 IS- 44|  
 PG- 5073-5082|

DA- 19 October, |  
 PY- 2000 |  
 ME- print |  
 SN- 0950-9232 |  
 DT- Article |  
 RT- Abstract |  
 LA- English |  
 SL- English |  
 AB- The transcriptional response of mouse pro-B cells to two different apoptotic stimuli was investigated. First, interleukin-3 (IL-3) deprivation was used to trigger programmed cell death in IL-3 dependent FL5.12 cells. Alternatively, cells were treated with the protein kinase C (PKC) inhibitor staurosporine. The temporal pattern of gene expression was followed with cDNA microarrays, covering over 8700 different mouse cDNA sequences corresponding to approximately 7900 unique genes. Messenger RNA levels of 315 genes were found to be regulated by more than twofold upon IL-3 removal, while 125 genes reacted to staurosporine treatment. Cross-comparison revealed an intersection of 34 genes similarly regulated in both pathways and thus representing candidates for common apoptosis regulators. For many expressed sequence tags (ESTs) our data suggest for the first time functions in the control of apoptosis, stress response or the cell cycle. IL-3 removal led to the repression of genes required for proliferation and to the induction of genes, linked to apoptotic and signaling pathways. Staurosporine caused predominantly activation of genes, some of which had previously been described to be involved in inflammation. Our findings indicate that cellular responses to both apoptotic stimuli influence various physiological pathways which had not previously been known to be linked. |  
 RN- 141436-78-4: PROTEIN KINASE C; 62996-74-1: STAUROSPORINE |  
 DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular Biophysics); Cell Biology; Methods and Techniques |  
 DE- <BIOSYSTEMATIC> Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia |  
 DE- <ORGANISMS> FL5.12 cell line (Muridae)--mouse pro-B cell |  
 DE- <SUPER TAXA> Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates |  
 DE- <CHEMICALS> cDNA {complementary DNA}; interleukin-3--deprivation; messenger RNA; protein kinase C--inhibition; staurosporine--enzyme inhibitor |  
 DE- <METHODS> comparative microarray analysis--analytical method |  
 DE- <MISC.> apoptosis; cell cycle; gene expression |  
 CC- 10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
 02502 Cytology and Cytochemistry-General  
 02506 Cytology and Cytochemistry-Animal  
 03502 Genetics and Cytogenetics-General  
 03506 Genetics and Cytogenetics-Animal  
 10060 Biochemical Studies-General  
 10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines  
 10802 Enzymes-General and Comparative Studies; Coenzymes |  
 BC- 86375 Muridae |

3/4/8 (Item 7 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R) |  
 CZ- (c) 2001 BIOSIS. All rts. reserv. |  
 AZ- 12771031 |  
 AA- 200000524654 |  
 TI- Amphotericin B-induced differential expression of genes encoding immunomodulatory proteins in human T- and B-lymphocytes detected by cDNA microarray. |  
 AU- Rogers P David(a); Pearson Margaret M(a); Chapman Stanley W(a); Sullivan Donna C(a); Cleary John D(a) |  
 CS- (a)University of Mississippi, Jackson, MS USA |  
 JN- Pharmacotherapy |  
 VO- 20 |  
 IS- 10 |  
 PG- 1262 |  
 DA- October,



PY- 2000|  
 ME- print|  
 CT- 2000 Annual Meeting of the American College of Clinical Pharmacy|  
 LO- Los Angeles, California, USA|  
 DA- November 03-08, 2000|  
 SN- 0277-0008|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 RN- 1397-89-3: AMPHOTERICIN B|  
 DE- <MAJOR CONCEPT> Biochemistry and Molecular Biophysics; Immune System  
 (Chemical Coordination and Homeostasis)|  
 DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata,  
 Animalia|  
 DE- <ORGANISMS> Jurkat cell line (Hominidae)--human T-cell; Raji cell line  
 (Hominidae)--human B-cell|  
 DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates  
 |  
 DE- <CHEMICALS> amphotericin B--immunomodulatory activity; antigen  
 presentation proteins; cDNA {complementary DNA}; cell adhesion  
 proteins; chemokines; complement function proteins; cytokine  
 receptors; cytokines|  
 DE- <MISC.> differential gene expression; Meeting Abstract|  
 CC- 10060 Biochemical Studies-General  
 00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals  
 02508 Cytology and Cytochemistry-Human  
 10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines  
 17002 Endocrine System-General  
 34502 Immunology and Immunochemistry-General; Methods|  
 BC- 86215 Hominidae|

3/4/9 (Item 8 from file: 5)

FN- DIALOG(R) File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12734698|  
 AA- 200000488321|  
 TI- Identification of polyribosomes-associated mRNAs regulated by fragile X  
 mental retardation protein (FMRP) using oligonucleotide microarrays  
 .|  
 AU- Jin P(a); Feng Y; Brown V(a); Warren S T(a)|  
 CS- (a)Department of Biochemistry, Pediatrics and Genetics, Howard Hughes  
 Medical Institute, Atlanta, GA USA|  
 JN- American Journal of Human Genetics|  
 VO- 67|  
 IS- 4 Supplement 2|  
 PG- 18|  
 DA- October,|  
 PY- 2000|  
 ME- print|  
 CT- 50th Annual Meeting of the American Society of Human Genetics|  
 LO- Philadelphia, Pennsylvania, USA|  
 DA- October 03-07, 2000|  
 SP- American Society of Human Genetics; |  
 SN- 0002-9297|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 DE- <MAJOR CONCEPT> Medical Genetics (Allied Medical Sciences); Psychiatry  
 (Human Medicine, Medical Sciences)|  
 DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata,  
 Animalia|  
 DE- <ORGANISMS> human (Hominidae)--male, patient|  
 DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates  
 |  
 DE- <DISEASES> fragile X syndrome--behavioral and mental disorders,  
 congenital disease|  
 DE- <CHEMICALS> fragile X mental retardation protein;

polyribosome-associated mRNA--regulation; human FMR-1 gene {human fragile X mental retardation gene} (Hominidae)|  
 DE- <METHODS> microarray analysis--detection method|  
 DE- <MISC.> Meeting Abstract|  
 DE- <ALT. INDEX> Fragile X Syndrome (MeSH)|  
 CC- 21002 Psychiatry-Psychopathology; Psychodynamics and Therapy  
 00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals  
 03508 Genetics and Cytogenetics-Human  
 07004 Behavioral Biology-Human Behavior  
 25503 Developmental Biology-Embryology-Pathological|  
 BC- 86215 Hominidae|

3/4/10 (Item 9 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12706478|  
 AA- 200000459980|  
 TI- Printing proteins as microarrays for high-throughput function  
 determination.|  
 AU- MacBeath Gavin(a); Schreiber Stuart L|  
 CS- (a)Center for Genomics Research, Harvard University, 16 Divinity  
 Avenue, Cambridge, MA, 02138 USA|  
 JN- Science (Washington D C)|  
 VO- 289|  
 IS- 5485|  
 PG- 1760-1763|  
 DA- 8 September,|  
 PY- 2000|  
 ME- print|  
 SN- 0036-8075|  
 DT- Article|  
 RT- Abstract|  
 LA- English|  
 SL- English|  
 AB- Systematic efforts are currently under way to construct defined sets of  
 cloned genes for high-throughput expression and purification of  
 recombinant proteins. To facilitate subsequent studies of protein  
 function, we have developed miniaturized assays that accommodate  
 extremely low sample volumes and enable the rapid, simultaneous  
 processing of thousands of proteins. A high-precision robot designed to  
 manufacture complementary DNA microarrays was used to spot proteins  
 onto chemically derivatized glass slides at extremely high spatial  
 densities. The proteins attached covalently to the slide surface yet  
 retained their ability to interact specifically with other proteins, or  
 with small molecules, in solution. Three applications for protein  
 microarrays were demonstrated: screening for protein-protein  
 interactions, identifying the substrates of protein kinases, and  
 identifying the protein targets of small molecules.|  
 RN- 9026-43-1: PROTEIN KINASES|  
 DE- <MAJOR CONCEPT> Biochemistry and Molecular Biophysics; Methods and  
 Techniques|  
 DE- <CHEMICALS> complementary DNA; protein kinases; proteins|  
 DE- <METHODS> protein microarrays--analytical method|  
 DE- <MISC.> protein-protein interactions|  
 CC- 10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines  
 10060 Biochemical Studies-General  
 10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
 10802 Enzymes-General and Comparative Studies; Coenzymes|

3/4/11 (Item 10 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12665273|  
 AA- 200000418775|  
 TI- Microarray-based identification of a novel Streptococcus pneumoniae  
 regulon controlled by an autoinduced peptide.

AU- de Saizieu Antoine; Gardes Christophe; Flint Nicholas; Wagner Christian  
; Kamber Markus; Mitchell Timothy J; Keck Wolfgang; Amrein Kurt E;  
Lange Roland(a)|

CS- (a)Hoffmann-La Roche Ltd., Grenzacherstrasse 124, Bldg. 70/4, CH-4070,  
Basel Switzerland|

JN- Journal of Bacteriology|

VO- 182|

IS- 17|

PG- 4696-4703|

DA- September,|

PY- 2000|

ME- print|

SN- 0021-9193|

DT- Article|

RT- Abstract|

LA- English|

SL- English|

AB- We have identified in the Streptococcus pneumoniae genome sequence a  
two-component system (TCS13, Blp (bacteriocin-like peptide)) which is  
closely related to quorum-sensing systems regulating cell  
density-dependent phenotypes such as the development of genetic  
competence or the production of antimicrobial peptides in lactic acid  
bacteria. In this study we present evidence that TCS13 is a  
peptide-sensing system that controls a regulon including genes encoding  
Blps. Downstream of the Blp TCS (BlpH R) we identified open reading  
frames (blpAB) that have the potential to encode an ABC transporter  
that is homologous to the ComA/B export system for the  
competence-stimulating peptide ComC. The putative translation product  
of blpC, a small gene located downstream of blpAB, has a leader peptide  
with a Gly-Gly motif. This leader peptide is typical of precursors  
processed by this family of transporters. Microarray-based expression  
profiling showed that a synthetic oligopeptide corresponding to the  
processed form of BlpC (BlpC\*) induces a distinct set of 16 genes. The  
changes in the expression profile elicited by synthetic BlpC\* depend on  
BlpH since insertional inactivation of its corresponding gene abolishes  
differential gene induction. Comparison of the promoter regions of the  
blp genes disclosed a conserved sequence element formed by two  
imperfect direct repeats upstream of extended -10 promoter elements. We  
propose that BlpH is the sensor for BlpC\* and the conserved sequence  
element is a recognition sequence for the BlpR response regulator.|

DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular  
Biophysics)|

DE- <BIOSYSTEMATIC> Gram-Positive Cocci--Eubacteria, Bacteria,  
Microorganisms|

DE- <ORGANISMS> Streptococcus pneumoniae (Gram-Positive Cocci)|

DE- <SUPER TAXA> Bacteria; Eubacteria; Microorganisms|

DE- <CHEMICALS> BlpC; BlpH R; Blps; ComA; Streptococcus pneumoniae genome;  
TCS13; blp gene promoters; blpAB; Streptococcus pneumoniae blpAB gene  
(Gram-Positive Cocci)|

DE- <MOLECULAR SEQUENCE DATABANK NO.> AJ276401--GenBank, nucleotide  
sequence|

DE- <METHODS> microarray-based identification--analytical method|

DE- <MISC.> gene expression|

CC- 03502 Genetics and Cytogenetics-General

10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines

31000 Physiology and Biochemistry of Bacteria

31500 Genetics of Bacteria and Viruses|

BC- 07700 Gram-Positive Cocci (1992- )|

3/4/12 (Item 11 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|

CZ- (c) 2001 BIOSIS. All rts. reserv.|

AZ- 12554795|

AA- 200000308297|

TI- Neuroendocrine expression in metastatic prostate cancer: Evaluation of  
high throughput tissue microarrays to detect heterogeneous protein  
expression.!

AU- Mucci Neil R; Akdas Guliz; Manely Sargum; Rubin Mark A|

CS- (a)Department of Pathology, University of Michigan, 1500 East Medical Center Drive, Room 2G332, Ann Arbor, MI, 48109-0054 USA|

JN- Human Pathology|

VO- 31|

IS- 4|

PG- 406-414|

DA- April,|

PY- 2000|

ME- print|

SN- 0046-8177|

DT- Article|

RT- Abstract|

LA- English|

SL- English|

AB- The theory that poorly differentiated prostate carcinoma develops a neuroendocrine (NE) phenotype is controversial. Supportive data is variable with NE expression being observed in anywhere from 5% to 83% of prostate cancers. These percentages are derived from standard immunohistochemistry studies, which make no attempt to quantify the results. High-density tissue microarrays (TMAs), represent a novel method for evaluating up to 1000 tissue samples with a 0.6 mm diameter on a single glass slide. This high throughput technology for screening antibodies, however, requires validation to determine if TMAs are useful in evaluating heterogeneously expressed proteins such as the NE markers chromogranin A (CGA) and synaptophysin (SYN). This study compares results from standard slides to TMAs in 50 primary and metastatic prostate tumors taken from 12 rapid autopsies from men with hormone refractory prostate cancer. One hundred standard and 2 TMA slides were immunostained for CGA and SYN. Using standard slides, focal NE expression was seen in 1/12 primary prostate tumors. Overall, 13/100 (13%) standard slides showed focal NE expression for both primary and metastatic prostate tumors; NE expression was observed in 4/12 autopsy cases (33%) when all tumor sites per case were considered. 458 tissue elements (tumor and normal) were arrayed into one paraffin block. Seventy-three percent (332/458) of the elements placed into the TMA were confirmed histologically to represent tumor. Seventy-five percent (250/332) and 66% (218/332) could be evaluated for CGA and SYN expression, respectively. Six of the metastatic tumors expressed CGA and SYN or 2.4% (6/250; 95% CI = 0.9% to 5.2%) and 2.3% (6/218; 95% CI = 0.8% to 5.3%), respectively. In conclusion, only focal NE expression was observed by both methods (eg, standard and TMA slides). The focal expression in these advanced prostate tumors was unexpected given data from prostate tumor cell lines and animal models suggesting that progression to the NE phenotype parallels tumor progression. This study also supports the use of high density TMAs to screen for protein expression, even when expression is focal.|

DE- <MAJOR CONCEPT> Oncology (Human Medicine, Medical Sciences); Methods and Techniques|

DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia|

DE- <ORGANISMS> human (Hominidae)--male|

DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates|

DE- <DISEASES> hormone refractory prostate cancer--metastasis, neoplastic disease, reproductive system disease/male, urologic disease|

DE- <CHEMICALS> chromogranin A--neuroendocrine marker; synaptophysin--neuroendocrine marker|

DE- <METHODS> high-density tissue microarrays--analytical method|

DE- <MISC.> neuroendocrine phenotype; protein expression|

CC- 24004 Neoplasms and Neoplastic Agents-Pathology; Clinical Aspects; Systemic Effects

10060 Biochemical Studies-General

10504 Biophysics-General Biophysical Techniques

15501 Urinary System and External Secretions-General; Methods

16501 Reproductive System-General; Methods

17020 Endocrine System-Neuroendocrinology (1972- )

20501 Nervous System-General; Methods|

BC- 96215 Hominidae|

3/4/13 (Item 12 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12544581|  
AA- 200000298083|  
TI- Microarrays to study the genetic basis of proteinuria (UPV).|  
AU- Kita Y; Shiozawa M; Majewski R R; Rangel A; Provoost A P; Jacob H J|  
CS- (a)Medical College of Wisconsin, Milwaukee, WI USA|  
JN- FASEB Journal|  
VO- 14|  
IS- 4|  
PG- A328|  
DA- March 15,|  
PY- 2000|  
ME- print|  
CT- Annual Meeting of Professional Research Scientists: Experimental  
Biology 2000|  
LO- San Diego, California, USA|  
DA- April 15-18, 2000|  
SP- Federation of American Societies for Experimental Biology; |  
SN- 0892-6638|  
RT- Citation|  
LA- English|  
SL- English|  
DE- <MAJOR CONCEPT> Urinary System (Chemical Coordination and Homeostasis);  
Genetics|  
DE- <BIOSYSTEMATIC> Muridae--Rodentia, Mammalia, Vertebrata, Chordata,  
Animalia|  
DE- <ORGANISMS> rat (Muridae)--fawn-hooded, strain-ACI|  
DE- <SUPER TAXA> Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman  
Vertebrates; Rodents; Vertebrates|  
DE- <DISEASES> proteinuria--genetic basis, urologic disease|  
DE- <MISC.> physiological genomics; Meeting Abstract|  
DE- <ALT. INDEX> Proteinuria (MeSH)|  
CC- 03506 Genetics and Cytogenetics-Animal  
10050 Biochemical Methods-General  
10060 Biochemical Studies-General  
15501 Urinary System and External Secretions-General; Methods  
10502 Biophysics-General Biophysical Studies  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals|  
BC- 86375 Muridae|

3/4/14 (Item 13 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12522220|  
AA- 200000275722|  
TI- Chemical microarray system to phenotype the protein kinase  
enzymatic profile of cell lines and tissues.|  
AU- Lam Kit S(a); Falsey James; Manat Renil; Park Steven|  
CS- (a)Arizona Cancer Ctr, Tucson, AZ USA|  
JN- Proceedings of the American Association for Cancer Research Annual  
Meeting|  
IS- 41|  
PG- 850|  
DA- March,|  
PY- 2000|  
ME- print.|  
CT- 91st Annual Meeting of the American Association for Cancer Research.|  
LO- San Francisco, California, USA|  
DA- April 01-05, 2000|  
SN- 0197-016X|  
RT- Citation|  
LA- English|  
SL- English|  
PN- 9026-42-1: PROTEIN KINASE|

DE- <MAJOR CONCEPT> Tumor Biology|  
 DE- <BIOSYSTEMATIC> Animalia|  
 DE- <ORGANISMS> animal (Animalia)--animal model|  
 DE- <SUPER TAXA> Animals|  
 DE- <DISEASES> cancer--in-vitro cell study, neoplastic disease, tissue sample study|  
 DE- <CHEMICALS> protein kinase--enzymatic profile phenotype, tumor cell activity, tumor tissue activity|  
 DE- <METHODS> chemical microarray system--analytical method, genetic method|  
 DE- <MISC.> Meeting Abstract|  
 DE- <ALT. INDEX> Neoplasms (MeSH)|  
 CC- 10802 Enzymes-General and Comparative Studies; Coenzymes  
 24004 Neoplasms and Neoplastic Agents-Pathology; Clinical Aspects; Systemic Effects  
 00520 General Biology-Symposia, Transactions and Proceedings of Conferences, Congresses, Review Annuals|  
 BC- 33000 Animalia-Unspecified|

3/4/15 (Item 14 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12521862|  
 AA- 200000275364|  
 TI- Activation of the ribosomal protein S6 kinase by 17q23 amplification in breast cancer with short survival - A cDNA and tissue microarray study.|  
 AU- Barlund Maarit T(a); Forozan Farahnaz; Kononen Juha T; Bubendorf Lukas; Chen Yidong; Bittner Michael L; Torhorst Joachim; Haas Philippe; Bucher Christopher; Sauter Guido; Kallioniemi Olli P; Kallioniemi Anne|  
 CS- (a)Nhgri, NIH, Bethesda, MD USA|  
 JN- Proceedings of the American Association for Cancer Research Annual Meeting|  
 IS- 41|  
 PG- 726|  
 DA- March,|  
 PY- 2000|  
 ME- print.|  
 CT- 91st Annual Meeting of the American Association for Cancer Research.|  
 LO- San Francisco, California, USA|  
 DA- April 01-05, 2000|  
 SN- 0197-016X|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 RN- 90698-26-3: S6 KINASE|  
 DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular Biophysics); Reproductive System (Reproduction); Tumor Biology|  
 DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia|  
 DE- <ORGANISMS> MCF-7 cell line (Hominidae)--human breast cancer cells|  
 DE- <PARTS,ETC> chromosome 17q23--gene amplification|  
 DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates|  
 DE- <DISEASES> breast cancer--neoplastic disease, reproductive system disease/female|  
 DE- <CHEMICALS> S6 kinase--activation, ribosomal protein|  
 DE- <METHODS> cDNA microarray analysis--genetic method; comparative-genomic hybridization--genetic method; tissue microarray techniques--cytogenetic method|  
 DE- <MISC.> Meeting Abstract|  
 DE- <ALT. INDEX> Breast Neoplasms (MeSH)|  
 CC- 24002 Neoplasms and Neoplastic Agents-General  
 02508 Cytology and Cytochemistry-Human  
 03508 Genetics and Cytogenetics-Human  
 10060 Biochemical Studies-General  
 10802 Enzymes-General and Comparative Studies; Coenzymes  
 16301 Reproductive System-General; Methods

10502 Biophysics-General Biophysical Studies  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals|  
BC- 86215 Hominidae|

3/4/16 (Item 15 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12481096|  
AA- 200000234598|  
TI- Gene microarray profiling of native peptide versus altered peptide  
ligand stimulated encephalitogenic T cells.|  
AU- Garren Hideki(a); Lock Christopher B(a); Steinman Lawrence(a)|  
CS- (a)Stanford, CA USA|  
JN- Neurology|  
VO- 54|  
IS- 7 Supp. 3|  
PG- A260|  
DA- April 11,|  
PY- 2000|  
CT- 52nd Annual Meeting of the American Academy of Neurology.|  
LO- San Diego, CA, USA|  
DA- April 29-May 06, 2000|  
SN- 0028-3878|  
RT- Citation|  
LA- English|  
SL- English|  
DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular  
Biophysics); Immune System (Chemical Coordination and Homeostasis);  
Nervous System (Neural Coordination)|  
DE- <BIOSYSTEMATIC> Muridae--Rodentia, Mammalia, Vertebrata, Chordata,  
Animalia|  
DE- <ORGANISMS> mouse (Muridae)--transgenic|  
DE- <SUPER TAXA> Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman  
Vertebrates; Rodents; Vertebrates|  
DE- <CHEMICALS> peptide--altered, gene microarray profiling, native|  
DE- <MISC.> experimental autoimmune encephalomyelitis--T cell tolerance;  
Meeting Abstract|  
CC- 20501 Nervous System-General; Methods  
02502 Cytology and Cytochemistry-General  
03502 Genetics and Cytogenetics-General  
34502 Immunology and Immunochemistry-General; Methods  
15001 Blood, Blood-Forming Organs and Body Fluids-General; Methods  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals|  
BC- 86375 Muridae|

3/4/17 (Item 16 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12476130|  
AA- 200000229632|  
TI- Transcriptional alterations induced by papillomavirus E2 protein:  
cDNA microarray analysis.|  
AU- Goodwin E C(a); Zimmermann J W; Jones D A; DiMaio D(a); Leachman S A|  
CS- (a)Yale University School of Medicine, New Haven, CT USA|  
JN- Journal of Investigative Dermatology|  
VO- 114|  
IS- 4|  
PG- 773|  
DA- April,|  
PY- 2000|  
CT- 61st Annual Meeting of the Society for Investigative Dermatology.|  
LO- Chicago, Illinois, USA|  
DA- May 10-14, 2000|  
SN- 0022-202X|  
RT- Citation

LA- English|  
 SL- English|  
 DE- <MAJOR CONCEPT> Immune System (Chemical Coordination and Homeostasis);  
 Integumentary System (Chemical Coordination and Homeostasis); Genetics|  
 DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata,  
 Animalia|  
 DE- <ORGANISMS> HeLa cell line (Hominidae)--human cells|  
 DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates  
 |  
 DE- <CHEMICALS> papillomavirus E2 protein--transcriptional alterations|  
 DE- <METHODS> complementary DNA microarray analysis--analytical method,  
 genetic method|  
 DE- <MISC.> Meeting Abstract|  
 CC- 03508 Genetics and Cytogenetics-Human  
 02508 Cytology and Cytochemistry-Human  
 10050 Biochemical Methods-General  
 33502 Virology-General; Methods  
 34502 Immunology and Immunochemistry-General; Methods  
 18501 Integumentary System-General; Methods  
 00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals|  
 BC- 86215 Hominidae|

3/4/18 (Item 17 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12474948|  
 AA- 200000228450|  
 TI- A microarray screen for BCC-enriched genes reveals BEG4, a gene that  
 encodes a novel Shh-inducible, proline-rich protein. |  
 AU- Horng L(a); Zhen H H(a); Patil N; Oro A E(a)|  
 CS- (a)School of Medicine, Stanford University, Stanford, CA USA|  
 JN- Journal of Investigative Dermatology|  
 VO- 114|  
 IS- 4|  
 PG- 763|  
 DA- April,|  
 PY- 2000|  
 CT- 61st Annual Meeting of the Society for Investigative Dermatology.|  
 LO- Chicago, Illinois, USA|  
 DA- May 10-14, 2000|  
 SN- 0022-202X|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular  
 Biophysics); Integumentary System (Chemical Coordination and  
 Homeostasis); Tumor Biology|  
 DE- <BIOSYSTEMATIC> Animalia; Hominidae--Primates, Mammalia, Vertebrata,  
 Chordata, Animalia|  
 DE- <ORGANISMS> animal (Animalia)--transgenic; human (Hominidae)|  
 DE- <PARTS,ETC> epithelium--integumentary system; hair follicle--  
 integumentary system; outer root sheath--integumentary system; skin--  
 integumentary system, regenerated|  
 DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates  
 |  
 DE- <DISEASES> basal cell carcinoma--integumentary system disease,  
 neoplastic disease|  
 DE- <CHEMICALS> Sonic hedgehog target genes; human BEG4 gene {human basal  
 cell carcinoma-enriched gene 4} (Hominidae)|  
 DE- <METHODS> DNA microarray--analytical method, genetic method|  
 DE- <MISC.> Meeting Abstract|  
 DE- <ALT. INDEX> Carcinoma, Basal Cell (MeSH)|  
 CC- 18501 Integumentary System-General; Methods  
 03508 Genetics and Cytogenetics-Human  
 24002 Neoplasms and Neoplastic Agents-General  
 00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals|



BC- 33000 Animalia-Unspecified  
86215 Hominidae|

3/4/19 (Item 18 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12427443|  
AA- 200000180945|  
TI- Simultaneous analysis of protein, bacterial, and viral antigens using  
a flow cytometric microarray immunosensor.|  
AU- Venkateswaran Kodumudi S(a); Langlois Richard G(a)|  
CS- (a)Biology and Biotechnology Research Program, Lawrence Livermore  
National Laboratory, 7000 East Avenue, L-452, Livermore, CA, 94551 USA|  
JN- Abstracts of Papers American Chemical Society|  
VO- 219|  
IS- 1-2|  
PG- ANYL 201|  
PY- 2000|  
CT- 219th Meeting of the American Chemical Society.|  
LO- San Francisco, California, USA|  
DA- March 26-30, 2000|  
SP- American Chemical Society; |  
SN- 0065-7727|  
RT- Citation|  
LA- English|  
SL- English|  
DE- <MAJOR CONCEPT> Chemistry; Methods and Techniques; Microbiology|  
DE- <BIOSYSTEMATIC> Endospore-forming Gram-Positives--Eubacteria, Bacteria,  
Microorganisms; Enterobacteriaceae--Facultatively Anaerobic  
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Leviviridae--  
Bacterial Viruses, Viruses, Microorganisms|  
DE- <ORGANISMS> Bacillus globigii (Endospore-forming Gram-Positives)--spore  
; Erwinia herbicola (Enterobacteriaceae)--vegetative; bacteriophage  
MS2 (Leviviridae)|  
DE- <SUPER TAXA> Bacteria; Bacterial Viruses; Eubacteria; Microorganisms;  
Viruses|  
DE- <CHEMICALS> bacterial antigens--simultaneous analysis; ovalbumin--  
simultaneous analysis; proteins--simultaneous analysis; viral  
antigens--simultaneous analysis|  
DE- <METHODS> flow cytometric microarray immunosensor; flow cytometry--  
analytical method|  
DE- <MISC.> Meeting Abstract|  
CC- 10050 Biochemical Methods-General  
10054 Biochemical Methods-Proteins, Peptides and Amino Acids  
10060 Biochemical Studies-General  
10504 Biophysics-General Biophysical Techniques  
34504 Immunology and Immunochemistry-Bacterial, Viral and Fungal  
34502 Immunology and Immunochemistry-General; Methods  
10064 Biochemical Studies-Proteins, Peptides and Amino Acids  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals|  
BC- 02704 Leviviridae (1993- )  
06702 Enterobacteriaceae (1992- )  
07810 Endospore-forming Gram-Positives (1992- )|

3/4/20 (Item 19 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12216307|  
AA- 199900511156|  
TI- Microarray analysis revealed rapid and diverse changes of gene  
expression in the kidney of mice protein-overloaded proteinuria  
model.|  
AU- Takenaka Masaru(a); Nagasawa Yasuyuki(a); Kaimori Junya(a); Matsuoka  
Yasuko(a); Imai Enyu(a); Hori Masatsugu(a)|  
CS- (a)Department of Internal Medicine and Therapeutics, Osaka Univ.  
Graduate School of Medicine, Suita, Osaka Japan|

JN- Journal of the American Society of Nephrology|  
 VO- 10|  
 IS- PROGRAM AND ABSTR. ISSUE|  
 PG- 562A|  
 DA- Sept.,|  
 PY- 1999|  
 CT- 32nd Annual Meeting of the American Society of Nephrology|  
 LO- Miami Beach, Florida, USA|  
 DA- November 1-8, 1999|  
 SP- American Society of Nephrology; |  
 SN- 1046-6673|  
 RT- Citation|  
 LA- English|  
 DE- <MAJOR CONCEPT> Molecular Genetics (Biochemistry and Molecular  
 Biophysics); Urinary System (Chemical Coordination and Homeostasis)|  
 DE- <BIOSYSTEMATIC> Muridae--Rodentia, Mammalia, Vertebrata, Chordata,  
 Animalia|  
 DE- <ORGANISMS> mouse (Muridae)--animal model|  
 DE- <PARTS,ETC> kidney--excretory system|  
 DE- <SUPER TAXA> Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman  
 Vertebrates; Rodents; Vertebrates|  
 DE- <DISEASES> proteinuria--protein-overloaded, urologic disease; renal  
 disease--urologic disease|  
 DE- <CHEMICALS> kidney androgen regulated protein; mRNA {messenger RNA}--  
 expression; smooth muscle alpha actin|  
 DE- <METHODS> microarray analysis--analytical method|  
 DE- <MISC.> gene expression; Meeting Abstract; Meeting Poster|  
 DE- <ALT. INDEX> Kidney Diseases (MeSH); Proteinuria (MeSH)|  
 CC- 15501 Urinary System and External Secretions-General; Methods  
 03506 Genetics and Cytogenetics-Animal  
 10060 Biochemical Studies-General  
 10502 Biophysics-General Biophysical Studies  
 00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals|  
 BC- 86375 Muridae|

3/4/21 (Item 20 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12148486|  
 AA- 199900443335|  
 TI- Screening for novel interferon induced antiviral proteins using  
 oligonucleotide microarrays. |  
 AU- de Veer M J(a); Der S(a); Zhou A(a); Silverman R H(a); Williams BRG(a)|  
 CS- (a)Dept. of Cancer Biology, Lerner Research Institute, Cleveland Clinic  
 Foundation, Cleveland, OH, 44195 USA|  
 JN- Journal of Interferon and Cytokine Research|  
 VO- 19|  
 IS- SUPPL. 1|  
 PG- S93|  
 DA- Sept.,|  
 PY- 1999|  
 CT- Meeting of the International Society for Interferon and Cytokine  
 Research with the participation of the European Cytokine Society|  
 LO- Paris, France|  
 DA- September 5-9, 1999|  
 SP- European Cytokine Society; |  
 SN- 1079-9907|  
 RT- Citation|  
 LA- English|  
 DE- <MAJOR CONCEPT> Biochemistry and Molecular Biophysics; Endocrine System  
 (Chemical Coordination and Homeostasis)|  
 DE- <BIOSYSTEMATIC> Animalia|  
 DE- <ORGANISMS> 293T cell line (Animalia)|  
 DE- <PARTS,ETC> immune system--immune system|  
 DE- <SUPER TAXA> Animals|  
 DE- <CHEMICALS> interferon; oligonucleotide microarrays; IFN-alpha {  
 interferon-alpha}; RNA; TNF-alpha {tumor necrosis factor-alpha};

IFN-regulated gene|  
DE- <MISC.> apoptosis; Meeting Abstract|  
CC- 10060 Biochemical Studies-General  
02506 Cytology and Cytochemistry-Animal  
03506 Genetics and Cytogenetics-Animal  
17002 Endocrine System-General  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals|  
BC- 33000 Animalia-Unspecified|

3/4/22 (Item 21 from file: 5)  
FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12122478|  
AA- 199900417327|  
TI- Novel chemistry for parallel synthesis of microarrays of  
oligonucleotides and peptides using photogenerated acids.|  
AU- Gao Xiaolian(a); LeProust Eric M(a); Pellois Jean Philippe(a); Yu  
Peilin(a); Zhang Hua(a); Wang Wei; Zhou Xiaochuan|  
CS- (a)Department of Chemistry, University of Houston, 4800 Calhoun Rd,  
Houston, TX, 77204-5641 USA|  
JN- Abstracts of Papers American Chemical Society|  
VO- 218|  
IS- 1-2|  
PG- MEDI 3|  
PY- 1999|  
CT- 218th National Meeting of the American Chemical Society, Parts 1 and 2|  
LO- New Orleans, Louisiana, USA|  
DA- August 22-26, 1999|  
SP- American Chemical Society; |  
SN- 0065-7727|  
RT- Citation|  
LA- English|  
DE- <MAJOR CONCEPT> Chemistry; Methods and Techniques|  
DE- <CHEMICALS> oligonucleotides--microarrays, synthesis; photogenerated  
acids--reagent|  
DE- <METHODS> chemical synthesis--Synthesis/Modification Techniques,  
synthetic method|  
DE- <MISC.> Meeting Abstract|  
CC- 10060 Biochemical Studies-General  
10050 Biochemical Methods-General  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals|

3/4/23 (Item 22 from file: 5)  
FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 11994187|  
AA- 199900274706|  
TI- Protein microarrays for gene expression and antibody screening.|  
AU- Lueking Angelika(a); Horn Martin(a); Eickhoff Holger(a); Buessow Konrad  
(a); Lehrach Hans(a); Walter Gerald(a)|  
CS- (a)Max Planck Institute for Molecular Genetics, Ihnestrass 73,  
D-14195, Berlin Germany|  
JN- Analytical Biochemistry|  
VO- 270|  
IS- 1|  
PG- 103-111|  
DA- May 15,|  
PY- 1999|  
SN- 0003-2697|  
DT- Article|  
RT- Abstract|  
LA- English|  
SL- English|  
AB- Proteins translate genomic sequence information into function, enabling  
biological processes. As a complementary approach to gene expression

profiling on cDNA microarrays, we have developed a technique for high-throughput gene expression and antibody screening on chip-size protein microarrays. Using a picking/spotting robot equipped with a new transfer stamp, protein solutions were gridded onto polyvinylidene difluoride filters at high density. Specific purified protein was detected on the filters with high sensitivity (250 amol or 10 pg of a test protein). On a microarray made from bacterial lysates of 92 human cDNA clones expressed in a microtiter plate, putative protein expressors could be reliably identified. The rate of false-positive clones, expressing proteins in incorrect reading frames, was low. Product specificity of selected clones was confirmed on identical microarrays using monoclonal antibodies. Cross-reactivities of some antibodies with unrelated proteins imply the use of protein microarrays for antibody specificity screening against whole libraries of proteins. Because this application would not be restricted to antigen-antibody systems, protein microarrays should provide a general resource for high-throughput screens of gene expression and receptor-ligand interactions.

- DE- <MAJOR CONCEPT> Methods and Techniques; Molecular Genetics (Biochemistry and Molecular Biophysics)|
- DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia|
- DE- <ORGANISMS> human (Hominidae)|
- DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates|
- DE- <CHEMICALS> antibodies--screening; antigen-antibody systems; cDNA { complementary DNA}--cloning; ligands; protein microarrays--analysis, applications; proteins--analysis|
- DE- <METHODS> antibody detection--Detection/Labeling Techniques, analytical method; antibody screening--Analysis/Characterization Techniques--CB, analytical method; image analysis--imaging method, imaging techniques; protein purification--Isolation/Purification Techniques--CB, purification method|
- DE- <MISC.> biological processes; gene expression--analysis; receptor-ligand interactions--analysis; robotics|
- CC- 10054 Biochemical Methods-Proteins, Peptides and Amino Acids
- 03502 Genetics and Cytogenetics-General
- 10050 Biochemical Methods-General
- 10064 Biochemical Studies-Proteins, Peptides and Amino Acids
- 10506 Biophysics-Molecular Properties and Macromolecules
- 34502 Immunology and Immunochemistry-General; Methods
- 13002 Metabolism-General Metabolism; Metabolic Pathways
- 10300 Replication, Transcription, Translation
- 10060 Biochemical Studies-General
- 10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines|
- BC- 86215 Hominidae|

3/4/24 (Item 23 from file: 5)

- FN- DIALOG(R)File 5:Biosis Previews(R)|
- CZ- (c) 2001 BIOSIS. All rts. reserv.|
- AZ- 11915142|
- AA- 199900161251|
- TI- Protein detection using conducting polymer microarrays. |
- AU- Lu W; Nguyen T A; Wallace Gordon G(a)|
- CS- (a)Intelligent Polymer Res. Inst., Univ. Wollongong, Northfields Avenue, Wollongong, NSW 2522 Australia|
- JN- Electroanalysis|
- VO- 10|
- IS- 16|
- PG- 1101-1107|
- DA- Nov.,|
- PY- 1998|
- SN- 1040-0397|
- DT- Article|
- RT- Abstract|
- LA- English|
- AB- An array of conducting polymer coated microelectrodes was employed as an amperometric detector to analyze a range of proteins. Using

conducting polymer coatings with different counterions incorporated, varying selectivity series have been obtained. Protein identification and quantification were performed using chemometric techniques such as principal component analysis (PCA), soft independent modelling of class analogy (SIMCA), ordinary least square (OLS) and partial least square (PLS). Using four different polymers, response patterns were obtained and classification of six proteins was achieved. Individual proteins in a two-component mixture were quantitatively analyzed with acceptable accuracy.

DE- <MAJOR CONCEPT> Biochemistry and Molecular Biophysics; Mathematical Biology (Computational Biology); Methods and Techniques|  
DE- <CHEMICALS> protein--analysis, detection|  
DE- <METHODS> conducting polymer microarrays--equipment; ordinary least square analysis--Analysis/Characterization Techniques--CB, mathematical method; partial least square analysis--Analysis/Characterization Techniques--CB, mathematical method; principal component analysis--Analysis/Characterization Techniques--CB, mathematical method; protein detection--Detection/Labeling Techniques, detection method; soft independent modeling of class analogy--Analysis/Characterization Techniques--CB, mathematical model|  
CC- 10060 Biochemical Studies-General  
04500 Mathematical Biology and Statistical Methods  
10050 Biochemical Methods-General  
10502 Biophysics-General Biophysical Studies|

3/4/25 (Item 1 from file: 16)

DIALOG(R)File 16:Gale Group PROMT(R)  
(c) 2001 The Gale Group. All rts. reserv.

08276786 Supplier Number: 69846280 (USE FORMAT 7 FOR FULLTEXT)  
**IBC Presents the First Conference Dedicated to Protein Microarray Technology.**

PR Newswire, p4379

Feb 2, 2001

Language: English Record Type: Fulltext

Document Type: Newswire; Trade

Word Count: 220

PUBLISHER NAME: PR Newswire Association, Inc.

INDUSTRY NAMES: BUS (Business, General); BUSN (Any type of business)

3/4/26 (Item 2 from file: 16)

DIALOG(R)File 16:Gale Group PROMT(R)  
(c) 2001 The Gale Group. All rts. reserv.

07919803 Supplier Number: 66188363 (USE FORMAT 7 FOR FULLTEXT)  
**Oxford Glycosciences and Cambridge Antibody Technology announce strategic alliance in protein microarrays. (Brief Article)**

BIOTECH Patent News, n9, pNA

Sept, 2000

ISSN: 0898-2813

Language: English Record Type: Fulltext

Article Type: Brief Article

Document Type: Newsletter; Professional Trade

Word Count: 717

PUBLISHER NAME: Biotech Patent News

COMPANY NAMES: \*Cambridge Antibody Technology Group PLC; Oxford GlycoSciences PLC

EVENT NAMES: \*389 (Alliances, partnerships)

GEOGRAPHIC NAMES: \*1USA (United States); 4EUUK (United Kingdom)

PRODUCT NAMES: \*2834000 (Pharmaceutical Preparations); 2834736

(Protein Preparations NEC)

INDUSTRY NAMES: AGRI (Agriculture, Fishing and Tobacco); BIO (Biotechnology); BUSN (Any type of business); DRUG (Pharmaceuticals and Cosmetics); LAW (Law)

SIC CODES: 2834 (Pharmaceutical preparations)

NAICS CODES: 325412 (Pharmaceutical Preparation Manufacturing)

SPECIAL FEATURES: LOB; COMPANY

3/4/27 (Item 3 from file: 16)  
DIALOG(R)File 16:Gale Group PROMT(R)  
(c) 2001 The Gale Group. All rts. reserv.

07799963 Supplier Number: 65156141 (USE FORMAT 7 FOR FULLTEXT)  
**Oxford GlycoSciences plc and Cambridge Antibody Technology Announce  
Strategic Alliance in Protein Microarrays.**  
PR Newswire, pNA  
Sept 12, 2000  
Language: English Record Type: Fulltext  
Document Type: Newswire; Trade  
Word Count: 800  
PUBLISHER NAME: PR Newswire Association, Inc.  
COMPANY NAMES: \*Cambridge Antibody Technology  
INDUSTRY NAMES: BUS (Business, General); BUSN (Any type of business)  
SPECIAL FEATURES: COMPANY

3/4/28 (Item 1 from file: 20)  
DIALOG(R)File 20:World Reporter  
(c) 2001 The Dialog Corporation. All rts. reserv.

15743361 (USE FORMAT 7 OR 9 FOR FULLTEXT)  
**Biosite(R) Diagnostics Reports Advances In Developing Protein Microarray  
Platform**  
PR NEWSWIRE  
March 22, 2001  
JOURNAL CODE: WPRW LANGUAGE: English RECORD TYPE: FULLTEXT  
WORD COUNT: 1061

SAN DIEGO, March 22 /PRNewswire/ -- Biosite Diagnostics Incorporated (Nasdaq: BSTE) today announced that it has developed a prototype for a protein microarray comprising 100 discrete antibody zones. This high throughput microarray utilizes the Company's proprietary microcapillary technology and is capable of identifying and quantifying up to 100 proteins in a sample within 15 minutes. The achievement will be highlighted today during a workshop to be conducted by Kenneth F. Buechler, Ph.D, Biosite's vice president of research and Gunars Valkirs, Ph.D., Biosite's vice president of research and development, during the IBC Protein Microarray Technology conference. The conference is being held from March 21 through March 23 in San Diego.

(Photo: <http://www.newscom.com/cgi-bin/prnh/20010322/LATH054> )  
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3/4/29 (Item 2 from file: 20)  
DIALOG(R)File 20:World Reporter  
(c) 2001 The Dialog Corporation. All rts. reserv.

12932716  
**Oxford GlycoSciences PLC: (NewsWeb) Company and Packard BioScience Company  
announced collaboration to apply respective technologies to develop  
protein biochips ("microarrays").**  
EXTTEL COMPANY NEWS  
September 19, 2000  
JOURNAL CODE: FEXT LANGUAGE: English RECORD TYPE: FULLTEXT  
WORD COUNT: 16

Company and Packard BioScience Company announced collaboration to apply respective technologies to develop protein biochips ("microarrays").

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3/4/30 (Item 1 from file: 34)  
FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
CZ- 2001 Inst for Sci Info. All rts. reserv.

AN- 09569205|  
GA- 421LT|  
TI- Protein chips - New microarrays show how proteins interact|  
LA- English|  
AU- Voss D|  
JN- TECHNOLOGY REVIEW  
2001, V104, N4, P35-35|  
SN- 0040-1692|  
PU- MASS INST TECHNOL, BUILDING W59, CAMBRIDGE, MA 02139 USA|  
PY- 2001|  
DT- EDITORIAL MATERIAL|  
PD- 20010500|  
NR- 0|  
SC- MULTIDISCIPLINARY SCIENCES|

3/4/31 (Item 2 from file: 34)

FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci|  
CZ- (c) 2001 Inst for Sci Info. All rts. reserv.|  
AN- 09565832|  
GA- 421CD|  
TI- Reverse phase protein microarrays which capture disease progression  
show activation of pro-survival pathways at the cancer invasion front|  
AV- ABSTRACT AVAILABLE|  
LA- English|  
AU- Paweletz CP; Charboneau L; Bichsel VE; Simone NL; Chen T; Gillespie JW;  
Emmert-Buck MR; Roth MJ; Petricoin EF; Liotta LA (REPRINT) |  
CS- NCI,Pathol Lab NIH,Bldg 10/Bethesda//MD/20892 (REPRINT); NCI,Pathol Lab  
NIH,Bethesda//MD/20892; US FDA,Ctr Biol Evaluat & Res Tissue Prote  
Unit,Bethesda//MD/20892; NCI,Canc Prevent Studies Branch  
NIH,Bethesda//MD/20892; NCI,Pathogenet Unit Pathol Lab  
NIH,Bethesda//MD/20892; NCI,Canc Genome Anat Project Off Director  
NIH,Bethesda//MD/20892; Georgetown Univ,Dept Chem,Washington//DC/20057|  
GL- USA|  
JN- ONCOGENE  
2001, V20, N16, P1981-1989|  
SN- 0950-9232|  
PU- NATURE PUBLISHING GROUP, HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE,  
ENGLAND|  
PY- 2001|  
DT- ARTICLE|  
PD- 20010412|  
NR- 39|  
SC- BIOCHEMISTRY & MOLECULAR BIOLOGY; ONCOLOGY; CELL BIOLOGY; GENETICS &  
HEREDITY|  
AB- Protein arrays are described for screening of molecular markers and  
pathway targets in patient matched human tissue during disease  
progression. In contrast to previous protein arrays that immobilize the  
probe, our reverse phase protein array immobilizes the whole repertoire  
of patient proteins that represent the state of individual tissue cell  
populations undergoing disease transitions. A high degree of  
sensitivity, precision and linearity was achieved, making it possible  
to quantify the phosphorylated status of signal proteins in human  
tissue cell subpopulations. Using this novel protein microarray we have  
longitudinally analysed the state of pro-survival checkpoint proteins  
at the microscopic transition stage from patient matched histologically  
normal prostate epithelium to prostate intraepithelial neoplasia (PIN)  
and then to invasive prostate cancer. Cancer progression was associated  
with increased phosphorylation of Akt ( $P < 0.04$ ), suppression of  
apoptosis pathways ( $P < 0.03$ ), as well as decreased phosphorylation of  
ERK ( $P < 0.01$ ). At the transition from histologically normal epithelium  
to PIN we observed a statistically significant surge in phosphorylated  
Akt ( $P < 0.03$ ) and a concomitant suppression of downstream apoptosis  
pathways which proceeds the transition into invasive carcinoma.|  
DE- Author Keywords: laser capture microdissection ; protein microarrays ;  
apoptosis ; Akt ; mitogen activated protein kinase ; tumor progression|  
ID- Keyword Plus(R): PROSTATIC INTRAEPITHELIAL NEOPLASIA;  
GLYCOGEN-SYNTHASE KINASE-3; NERVE GROWTH-FACTOR; PROTEOMIC ANALYSIS;  
GENE-EXPRESSION; APOPTOSIS; AKT; IMMUNOASSAY; SPECIFICITY; ARRAYS;

CR- ARENKOV P, 2000, V278, P123, ANAL BIOCHEM  
 BOSTWICK DG, 1995, V75, P1823, CANCER  
 BUCKHOLZ RG, 1999, V1, P135, J MOL MICROBIOL BIOT  
 CROSS DAE, 1995, V378, P785, NATURE  
 DERISI J, 1996, V4, P457, NAT GENET  
 DIMMELER S, 2000, V86, P4, CIRC RES  
 EKINS RP, 1991, V37, P1955, CLIN CHEM  
 EMILI AQ, 2000, V18, P393, NAT BIOTECHNOL  
 EMMERTBUCK MR, 2000, V27, P158, MOL CARCINOGEN  
 EMMERTBUCK MR, 1996, V274, P1481, SCIENCE  
 ENGLERT CR, 1999, V60, P1526, CANCER RES  
 FODOR SPA, 1991, V251, P767, SCIENCE  
 FRANKE TF, 1997, V88, P435, CELL  
 FREY RS, 1997, V57, P628, CANCER RES  
 GE H, 2000, V28, P3, NUCLEIC ACIDS RES  
 GIOELI D, 1999, V59, P279, CANCER RES  
 GRAFF JR, 2000, V275, P24500, J BIOL CHEM  
 HAAB B, 2001, V2, P1, GENOME BIOL  
 HANAHAN D, 2000, V100, P57, CELL  
 JONES VW, 1998, V70, P1233, ANAL CHEM  
 KOCH M, 2000, V436, P413, VIRCHOWS ARCH  
 KONONEN J, 1998, V4, P844, NAT MED  
 KRAJEWSKA M, 1996, V1148, P1567, AM J PATHOL  
 LIPSHUTZ RJ, 1999, V1, P20, NAT GENET S  
 LUEKING A, 1999, V270, P103, ANAL BIOCHEM  
 MACBEATH G, 1999, V121, P7966, J AM CHEM SOC  
 MARSHALL CJ, 1995, V80, P179, CELL  
 ORNSTEIN DK, 2000, V21, P2235, ELECTROPHORESIS  
 PAGE MJ, 2000, V96, P12589, PNAS  
 PAP M, 1998, V273, P19929, J BIOL CHEM  
 PAWELETZ CP, 2000, V60, P6293, CANCER RES  
 ROWE CA, 1999, V71, P3846, ANAL CHEM  
 SCHENA M, 1995, V270, P467, SCIENCE  
 WILLIAMS KL, 1997, P1, PROTEOME RES NEW FRO  
 XIE W, 2000, V44, P31, PROSTATE  
 YAO R, 1995, V267, P2003, SCIENCE  
 YORK RD, 1998, V392, P622, NATURE  
 ZHENG DQ, 2000, V275, P24565, J BIOL CHEM  
 ZIMMERMANN S, 1999, V286, P1741, SCIENCE||

3/4/32 (Item 3 from file: 34)

FN- DIALOG(R) File 34:SciSearch(R) Cited Ref Sci|  
 CZ- (c) 2001 Inst for Sci Info. All rts. reserv.|  
 AN- 09535339|  
 GA- 418BF|  
 TI- Protein microarrays : prospects and problems|  
 AV- ABSTRACT AVAILABLE|  
 LA- English|  
 AU- Kodadek T (REPRINT) |  
 CS- Univ Texas,SW Med Ctr Dept Internal Med Ctr Biomed Invent,5323 Harry  
 Hines Blvd/Dallas//TX/75390 (REPRINT); Univ Texas,SW Med Ctr Dept  
 Internal Med Ctr Biomed Invent,Dallas//TX/75390; Univ Texas,SW Med Ctr  
 Dept Biochem Ctr Biomed Invent,Dallas//TX/75390|  
 GL- USA|  
 JN- CHEMISTRY & BIOLOGY  
 2001, V8, N2, P105-115|  
 SN- 1074-5521|  
 PU- CURRENT BIOLOGY LTD, 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND|  
 PY- 2001|  
 DT- ARTICLE|  
 PD- 20010200|  
 NR- 38|  
 SC- BIOCHEMISTRY & MOLECULAR BIOLOGY|  
 AB- Protein microarrays are potentially powerful tools in biochemistry and  
 molecular biology. Two types of protein microarrays are defined. One,  
 termed a protein function array, will consist of thousands of native  
 proteins immobilized in a defined pattern. Such arrays can be utilized  
 for massively parallel testing of protein function, hence the name. The



other type is termed a protein-detecting array. This will consist of large numbers of arrayed protein-binding agents. These arrays will allow for expression profiling to be done at the protein level. In this article, some of the major technological challenges to the development of protein arrays are discussed, along with potential solutions. (C) 2001 Elsevier Science Ltd. All rights reserved. |

- DE- Author Keywords: protein array ; microarray ; protein-protein interaction ; protein immobilization ; surface plasmon resonance |
- ID- Keyword Plus(R): CELL-CYCLE INHIBITOR; PHAGE DISPLAY; COMBINATORIAL LIBRARIES; BINDING; AFFINITY; PEPTIDES; SELECTION; YEAST; ANTIBODIES; PROTEOMICS |
- CR- ALBERTS B, 1998, V92, P291, CELL  
BARTEL PL, 1995, V254, P241, METHOD ENZYMOL  
BITTNER M, 2000, V406, P536, NATURE  
BODER ET, 2000, V97, P10701, P NATL ACAD SCI USA  
CLARK EA, 2000, V406, P532, NATURE  
COHEN BA, 1998, V95, P14272, P NATL ACAD SCI USA  
COLAS P, 1996, V380, P548, NATURE  
DEKRUIF J, 1996, V271, P7630, J BIOL CHEM  
DEWILDT RMT, 2000, V18, P989, NAT BIOTECHNOL  
DONG DL, 1999, V6, P133, CHEM BIOL  
FABBRIZIO E, 2000, V18, P4357, ONCOGENE  
FAIRBROTHER WJ, 1998, V37, P17754, BIOCHEMISTRY-US  
FORMOSA T, 1984, V49, P363, COLD SPRING HARB SYM  
GEORGIU G, 1997, V15, P29, NAT BIOTECHNOL  
GRIFFITHS AD, 1998, V9, P102, CURR OPIN BIOTECH  
GYGI SP, 1999, V17, P994, NAT BIOTECHNOL  
GYGI SP, 1999, V19, P1720, MOL CELL BIOL  
GYGI SP, 2000, V97, P9390, P NATL ACAD SCI USA  
HAN Y, 2000, V275, P14979, J BIOL CHEM  
HEGDE P, 2000, V29, P548, BIOTECHNIQUES  
HESSELBERTH J, 2000, V74, P25, REV MOL BIOTECHNOL  
HYDEDERUYSCHER R, 2000, V7, P17, CHEM BIOL  
LASHKARI DA, 1997, V94, P13057, P NATL ACAD SCI USA  
LECITRA EJ, 1996, V93, P12817, P NATL ACAD SCI USA  
LIPSHUTZ RJ, 1999, V21, P20, NAT GENET S  
MACBEATH G, 2000, V289, P1760, SCIENCE  
MALY DJ, 2000, V97, P2419, P NATL ACAD SCI USA  
MARTZEN MR, 1999, V286, P1153, SCIENCE  
NELSON RW, 2000, V21, P1155, ELECTROPHORESIS  
PANDEY A, 2000, V405, P837, NATURE  
PHIZICKY EM, 1995, V59, P94, MICROBIOL REV  
RADER C, 1997, V8, P503, CURR OPIN BIOTECH  
RICH RL, 2000, V11, P54, CURR OPIN BIOTECH  
SHUKER SB, 1996, V274, P1531, SCIENCE  
TSAO KL, 1996, V169, P59, GENE  
TUERK C, 1990, V249, P505, SCIENCE  
ZHANG ZW, 2000, V18, P71, NAT BIOTECHNOL  
ZHU WG, 2000, V275, P32098, J BIOL CHEM |

3/4/33 (Item 4 from file: 34)

- FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci |
- CZ- (c) 2001 Inst for Sci Info. All rts. reserv. |
- AN- 09147866 |
- GA- 372HR |
- TI- Protein expression profiling on microarrays by rolling circle amplification. |
- LA- English |
- AU- Schweitzer B; Wiltshire S; Lambert J; Mullenix M; Kingsmore S |
- JN- CLINICAL CHEMISTRY  
2000, V46, N11, P21-21 |
- SN- 0009-9147 |
- PU- AMER ASSOC CLINICAL CHEMISTRY, 2101 L STREET NW, SUITE 202, WASHINGTON, DC 20037-1526 |
- PY- 2000 |
- DT- MEETING ABSTRACT |
- PD- 20001100 |
- NR- 0 |

SF- CC LIFE--Current Contents, Life Sciences|  
SC- MEDICAL LABORATORY TECHNOLOGY|

3/4/34 (Item 5 from file: 34)  
FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci|  
CZ- (c) 2001 Inst for Sci Info. All rts. reserv.|  
AN- 09062021|  
GA- 362LR|  
TI- A protein microarray |  
LA- English|  
AU- Feng HP|  
JN- NATURE STRUCTURAL BIOLOGY  
2000, V7, N10, P829-829|  
SN- 1072-8368|  
PU- NATURE AMERICA INC, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707|  
PY- 2000|  
DT- EDITORIAL MATERIAL|  
PD- 20001000|  
NR- 0|  
SF- CC LIFE--Current Contents, Life Sciences|  
SC- BIOPHYSICS; BIOCHEMISTRY & MOLECULAR BIOLOGY; CELL BIOLOGY|

3/4/35 (Item 6 from file: 34)  
FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci|  
CZ- (c) 2001 Inst for Sci Info. All rts. reserv.|  
AN- 08990817|  
GA- 353PF|  
TI- Protein microarrays hit the spot|  
LA- English|  
AU- Borman S|  
JN- CHEMICAL & ENGINEERING NEWS  
2000, V78, N37, P6-7|  
SN- 0009-2347|  
PU- AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036|  
PY- 2000|  
DT- NEWS ITEM|  
PD- 20000911|  
NR- 3|  
SC- ENGINEERING, CHEMICAL|  
CR- MACBEATH G, 1999, V121, P7967, J AM CHEM SOC  
MACBEATH G, 2000, V122, P7849, J AM CHEM SOC  
SCHREIBER SL, 2000, V289, P1760, SCIENCE||

3/4/36 (Item 7 from file: 34)  
FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci|  
CZ- (c) 2001 Inst for Sci Info. All rts. reserv.|  
AN- 08751538|  
GA- 325TH|  
TI- Neuroendocrine expression in metastatic prostate cancer: Evaluation of  
high throughput tissue microarrays to detect heterogeneous protein  
expression (vol 31, pg 406, 2000)|  
LA- English|  
AU- Mucci|  
JN- HUMAN PATHOLOGY  
2000, V31, N6, P778-778|  
SN- 0046-8177|  
PU- W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300,  
PHILADELPHIA, PA 19106-3399|  
PY- 2000|  
DT- CORRECTION, ADDITION|  
PD- 20000600|  
NR- 1|  
SF- CC LIFE--Current Contents, Life Sciences; CC CLIN--Current Contents,  
Clinical Medicine|  
SC- PATHOLOGY|  
CR- MUCCI, 2000, V31, P406, HUM PATHOL

3/4/37 (Item 8 from file: 34)

FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci|  
CZ- (c) 2001 Inst for Sci Info. All rts. reserv.|  
AN- 07997132|  
GA- 234EG|  
TI- Printing small molecules as **microarrays** and detecting protein  
-ligand interactions en masse|  
LA- English|  
AU- MacBeath G; Koehler AN; Schreiber SL (REPRINT) |  
CS- HARVARD UNIV,DEPT CHEM & CHEM BIOL, CTR GENOM RES, HOWARD HUGHES MED  
INST, 12 OXFORD ST /CAMBRIDGE//MA/02138 (REPRINT); HARVARD UNIV,DEPT  
CHEM & CHEM BIOL, CTR GENOM RES, HOWARD HUGHES MED  
INST/CAMBRIDGE//MA/02138; HARVARD UNIV,HOWARD HUGHES MED INST, INST  
CHEM & CELL BIOL/CAMBRIDGE//MA/02138|  
GL- USA|  
JN- JOURNAL OF THE AMERICAN CHEMICAL SOCIETY  
1999, V121, N34, P7967-7968|  
SN- 0002-7863|  
PU- AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036|  
PY- 1999|  
DT- ARTICLE|  
PD- 19990901|  
NR- 8|  
SF- CC PHYS--Current Contents, Physical, Chemical & Earth Sciences; CC LIFE  
--Current Contents, Life Sciences|  
SC- CHEMISTRY|  
ID- KeyWord Plus(R): GENERAL-METHOD; LIBRARY|  
CR- CHAIET L, 1964, V106, P1, ARCH BIOCHEM BIOPHYS  
FURKA A, 1991, V37, P487, INT J PEPT PROT RES  
HOLT DA, 1993, V115, P9925, J AM CHEM SOC  
LAM KS, 1997, V97, P411, CHEM REV  
LAM KS, 1991, V354, P82, NATURE  
NESTLER HP, 1994, V59, P4723, J ORG CHEM  
SCHENA M, 1995, V270, P467, SCIENCE  
TAN DS, 1998, V120, P8565, J AM CHEM SOC||

3/4/38 (Item 9 from file: 34)

FN- DIALOG(R)File 34:SciSearch(R) Cited Ref Sci|  
CZ- (c) 2001 Inst for Sci Info. All rts. reserv.|  
AN- 07495706|  
GA- 173NX|  
TI- Create a **protein microarray** using a hydrogel "stamper"|  
AV- ABSTRACT AVAILABLE|  
LA- English|  
AU- Gaber BP (REPRINT) ; Martin BD; Turner DC|  
CS- USN,RES LAB, LAB MOL INTERFACIAL INTERACT/WASHINGTON//DC/20375  
(REPRINT); GEORGE MASON UNIV,CTR COMP SCI & INFORMAT/FAIRFAX//VA/22030;  
USN,RES LAB, CTR BIOMOL SCI & ENGN/WASHINGTON//DC/20375|  
GL- USA|  
JN- CHEMTECH  
1999, V29, N3, P20-24|  
SN- 0009-2703|  
PU- AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036|  
PY- 1999|  
DT- ARTICLE|  
PD- 19990300|  
NR- 34|  
SF- CC PHYS--Current Contents, Physical, Chemical & Earth Sciences; CC ENGI  
--Current Contents, Engineering, Computing & Technology; |  
SC- CHEMISTRY, APPLIED|  
AB- The keys to fabricating multiplexed biosensors are the capability to  
lay down biomolecules with spatial fidelity retention of activity, and  
an absolute minimum of biochemical cross-talk. The hydrogel stamper  
does this by delivering a molecular film of protein directly onto the  
surface in a single step.|  
ID- KeyWord Plus(R): SURFACES; DNA; FABRICATION; BIOSENSORS;

ANTIBODIES; CELLS; GOLD|  
 CR- BAINS W, 1988, V135, P303, J THEOR BIOL  
 BAKER DA, 1988, V29, P691, POLYMER  
 BHATIA SK, 1992, V114, P4432, J AM CHEM SOC  
 BRITLAND S, 1992, V8, P155, BIOTECHNOL PROGR  
 BURNS MA, 1998, V282, P484, SCIENCE  
 BYFIELD MP, 1994, V9, P373, BIOSENS BIOELECTRON  
 CHEE M, 1996, V227, P610, SCIENCE  
 CHEN X, 1995, V28, P15, CARBOHYD POLYM  
 CHRISSEY LA, 1996, V24, P3040, NUCLEIC ACIDS RES  
 DAVIES DR, 1990, V59, P439, ANNU REV BIOCHEM  
 DONTA N, 1997, V69, P2619, ANAL CHEM  
 FERGUSON JA, 1996, V14, P1681, NAT BIOTECHNOL  
 FODOR SPA, 1991, V251, P767, SCIENCE  
 GUSCHIN D, 1997, V250, P203, ANAL BIOCHEM  
 JANATA J, 1989, PCH1, PRINCIPLES CHEM SENS  
 KAZANSKI KS, 1993, V104, P97, ADV POLYM SCI  
 KIMURA J, 1988, V4, P41, BIOSENSORS  
 KUMAR A, 1993, V63, P2002, APPL PHYS LETT  
 LI RH, 1996, V50, P365, BIOTECHNOL BIOENG  
 LOPEZ GP, 1993, V115, P5877, J AM CHEM SOC  
 LU B, 1996, V121, PR29, ANALYST  
 LUNSTROM I, 1987, P201, POLYM SURFACES INTER  
 MARIUZZA RA, 1987, V16, P139, ANNU REV BIOPHYS BIO  
 MARKOWITZ MA, 1997, V68, P57, APPL BIOCHEM BIOTECH  
 MARTIN BD, 1998, V19, P69, BIOMATERIALS  
 MARTIN BD, 1998, V14, P3971, LANGMUIR  
 MARTIN BD, 1992, V25, P7081, MACROMOLECULES  
 PADDLE BM, 1996, V11, P1079, BIOSENS BIOELECTRON  
 PEPPAS NA, 1986, HYDROGELS MED PHARM  
 PRITCHARD DJ, 1995, V67, P3605, ANAL CHEM  
 ROGERS KR, 1996, V30, P486, ENVIRON SCI TECHNOL  
 SEFTON MV, 1993, V107, P143, ADV POLYM SCI  
 SINGHVI R, 1994, V264, P696, SCIENCE  
 WEN AC, 1996, V50, P357, BIOTECHNOL BIOENG|

3/4/39 (Item 1 from file: 71)  
 DIALOG(R)File 71:ELSEVIER BIOBASE  
 (c) 2001 Elsevier Science B.V. All rts. reserv.

AZ- 01486199|  
 AA- 2000158633|  
 TI- Erratum: Neuroendocrine expression in metastatic prostate cancer:  
 Evaluation of high throughput tissue **microarrays** to detect  
 heterogeneous **protein** expression (Human Pathology (April 2000) 31  
 (406-414))|  
 AU- Mucci|  
 DT- Journal|  
 JN- Human Pathology|  
 SO- Journal:Human Pathology ,31/6 (778) ,2000, United States|  
 CP- United States|  
 PY- 2000|  
 CD- HPCQA|  
 SN- 0046-8177|  
 DT- Erratum|  
 LA- English|  
 SH- 87.2.9.2 - CANCER RESEARCH TUMOUR BIOLOGY Genetics Gene expression  
 87.2.5 - CANCER RESEARCH TUMOUR BIOLOGY Invasion and Metastasis  
 87.5.16 - CANCER RESEARCH CLINICAL INVESTIGATIONS BY ORGAN SITE  
 Prostate|  
 HD- COMPLETED RECORD - July 27, 2000

3/4/40 (Item 1 from file: 73)  
 FN- DIALOG(R)File 73:EMBASE|  
 CZ- (c) 2001 Elsevier Science B.V. All rts. reserv.|  
 AZ- 11011719|  
 AA- <EMBASE> 2001051263|

TI- Advances in protein microarray technology for protein expression  
 and interaction profiling|  
 AU- Haab B.B.|  
 CS- B.B. Haab, Van Andel Institute, 333 Bostwick NE, Grand Rapids, MI 49503  
 |  
 CS- <COUNTRY> United States|  
 EL- brian.haab@vai.org|  
 JN- Current Opinion in Drug Discovery and Development ( CURR. OPIN. DRUG  
 DISCOV. DEV. )|  
 SO- 4/1 (116-123)|  
 PD- 2001|  
 PY- 2001|  
 SN- 1367-6733|  
 CP- United Kingdom|  
 CD- CODDF|  
 DT- <ITEM TYPE> Review|  
 DT- Journal|  
 LA- ENGLISH|  
 SL- ENGLISH|  
 AB- Protein microarrays address a great demand for high-throughput protein  
 analysis techniques. Because protein microarrays detect many proteins  
 in parallel, are quantitative, and have minimal reagent and sample  
 consumption requirements due to miniaturization, they are potentially  
 powerful tools for applications in basic and applied biology. Advances  
 in manufacturing, protein immobilization and detection methods have  
 enabled high-throughput protein quantitation and interaction studies.  
 Protein microarrays can be applied to protein function studies,  
 screening the production of antibodies and recombinant proteins,  
 discovery of proteins implicated in disease or that are potential drug  
 targets, and rapid detection or diagnosis of disease. A remaining  
 challenge for the full implementation of protein microarrays is the  
 acquisition of large sets of high-affinity and highly specific protein  
 capture reagents.|  
 RF- 39|  
 DE- <MAJOR DRUG TERM> protein|  
 DE- <MAJOR MEDICAL TERM> fluorescence; protein analysis; electronics|  
 DE- <MINOR MEDICAL TERM> protein expression; protein immobilization; drug  
 manufacture; screening; DNA microarray; review|  
 SH- 029 Clinical and Experimental Biochemistry|  
 RN- 67254-75-5 (protein)|

3/4/41 (Item 1 from file: 88)  
 DIALOG(R)File 88:Gale Group Business A.R.T.S.  
 (c) 2001 The Gale Group. All rts. reserv.

05511136 SUPPLIER NUMBER: 64824920  
 Microarray-based identification of a novel *Streptococcus pneumoniae*  
 regulon controlled by an autoinduced peptide.  
 Saizieu, Antoine de; Gardes, Christophe; Flint, Nicholas; Wagner, Christan;  
 Kamber, Markus; Mitchell, Timothy J.; Keck, Wolfgang; Amrein, Kurt E.;  
 Lange, Roland  
 Journal of Bacteriology, 182, 17-18, 4696(8)  
 Sept, 2000  
 ISSN: 0021-9193 LANGUAGE: English RECORD TYPE: Abstract

ABSTRACT: Research demonstrates a two-component system of bacteriocin-like  
 peptide that controls its own genes and a regulon. Data indicate that the  
 peptide is part of a quorum-sensing regulon which shares many features of  
 competence-stimulating peptide controlled regulon.

3/4/42 (Item 1 from file: 94)  
 FN- DIALOG(R)File 94:JICST-EPlus|  
 CZ- (c)2001 Japan Science and Tech Corp(JST). All rts. reserv.|  
 AN- 04739920|  
 AA- <JICST ACCESSION NUMBER> 01A0260420|  
 FS- PreJICST-E|  
 TI- Behaviors of Life Molecules. (74): Development of PNA(peptide nucleic

acid) with a linker on its strand for new microarray system.  
 AU- YASUDA KYOKO (1); SUGIMOTO NAOKI (1);  
 CS- (1) Konan Univ.  
 JN- Nippon Kagakkai Koen Yokoshu  
 SO- <PUBLICATION YEAR> 2000  
 SO- <VOLUME, ISSUE NBR> VOL.78th,NO.2  
 SO- <PAGE(S)> PAGE.819  
 JC- S0493AAY  
 SN- 0285-7626  
 LA- Japanese  
 DT- Conference Proceeding  
 MT- Printed Publication  
 CP- Japan  
 AB- PNA(peptide nucleic acid) is an analogue of DNA in which natural DNA backbone is replaced by N-(aminoethyl)glycine unit. We investigated recognition ability of PNA with a linker on its strand by using SPR(Surface Plasmon Resonance). The result showed that PNA (ATAAATTG-D-GATACAAA), where D is delta omega amino acid, have a greater DNA recognition ability than normal PNA. We will also report optimum length of omega amino acid. (author abst.)

3/4/43 (Item 1 from file: 98)

DIALOG(R)File 98:General Sci Abs/Full-Text  
 (c) 2001 The HW Wilson Co. All rts. reserv.

AN- 04389682  
 AA- BGSA00139682  
 TI- Protein microarrays.  
 JN- Analytical Chemistry  
 JN- Anal Chem  
 PD- Nov. 1 2000 (20001101)  
 SF- il  
 SN- 0003-2700  
 LA- English  
 CP- United States  
 RT- Abstract  
 ST- Corrected or revised record  
 AB- Researchers at Harvard University, Massachusetts, have applied new DNA microarray technology to proteins. MacBeath and Schreiber used their adapted system, reported in a recent Science (2000;289:1760-3), to assay protein-protein interactions, to screen for enzyme substrates, and to study protein-small molecule experiments.  
 DE- Proteins--Analysis DNA chips

3/4/44 (Item 1 from file: 107)

FN- DIALOG(R)File 107:Adis R&D Insight  
 CZ- (c) 2001 Adis International Ltd. All rts. reserv.

AN- 00108572  
 AZ- 00108572  
 AA- 015164  
 RD- 20010118  
 NA- Research programme: cardiovascular disease - Incyte Genomics/  
 University of Maastricht  
 AC- C Cardiovascular System  
 CC- C6A Other Cardiovascular Products  
 ME- Undefined mechanism  
 LO- Nonindustrial source (Netherlands); Incyte Genomics (USA)  
 IP- Incyte Genomics; Nonindustrial source  
 DP- Preclinical  
 ST- Preclinical, USA, Cardiovascular disorders  
 IT- Introduction:

Incyte Genomics and The Cardiovascular Research Institute Maastricht at the University of Maastricht (CARIM) in the Netherlands have entered into a genomic research partnership to study cardiovascular disease. Intellectual property resulting from this collaboration will be owned jointly by CARIM and Incyte and available for licensing through Incyte. Scientists at CARIM and Incyte will design and conduct gene expression

experiments to study the role genes play in the prevention, diagnosis and treatment of cardiovascular disease. Researchers at CARIM will gain access to Incyte's lifeseq sup((R)) gold gene sequence database and Incyte's lifeexpress sup(TM) expression database for internal use. In return, CARIM will provide clinical and experimental samples for analysis. Data from the collaboration will be incorporated into lifeexpress sup(TM) for scientists to explore and correlate gene and protein behaviour in diseased and normal tissues, prioritise potential drug targets and assess the efficacy and toxicity of compounds. Treatments for cardiovascular disease are currently limited. According to the scientific director of CARIM, access to Incyte's integrated genomics platform, including gene expression information and advanced microarray technology will further our understanding of the genetic basis of cardiovascular disease and accelerate efforts to identify effective approaches for the early diagnosis, prevention and treatment of cardiovascular disease.

PO- Mechanism of action:  
Undefined mechanism|

UP- 18-Jan-2001: New profile

18-Jan-2001: Preclinical development for Cardiovascular disorders in USA (Unknown route)|

3/4/45 (Item 1 from file: 115)

FN- DIALOG(R)File 115:Research Centers & Services|

CZ- (c) 2000 Gale Research Inc. All rts. reserv.|

AN- <Accession Nbr> 09998238|

AN- <Source Book> Research Centers Directory|RCD|

AN- <Internal No> 1000224600|

ON- <Org. Name> Pennsylvania State University|Biotechnology Institute|

AD- <Street> 519 Wartik Laboratory|

AD- <Last> University Park, PA 16802 USA|

OF- Dr. Nina V. Fedoroff, Dir.|

TE- <Phone> (814)863-3650|

FX- (814)863-1357|

EL- nvf1@email.psu.edu|

UR- http://www.lsc.psu.edu|

AB- <OC> FOUNDED: 1984. DESCRIPTION: Integral unit of the Life Sciences Consortium at Pennsylvania State University. RESEARCH BUDGET: US\$4,945,716. REVENUES: US\$399,000. STAFF: 9 Support, 1 Administrative, 14 Other. FINANCIAL SUPPORT: Parent institution, industry, U.S. government, state government, individual gifts.|

AB- <RD> RESEARCH: Plant biotechnology ranging from research in plant biochemistry and molecular biology to plant development. RESOURCES: Electron Microscope Facility; Center of Computational Biology; Hybridomaa and Cell Culture Laboratory; Center for Quantitative Cell Analysis, and the Nucleic Acid Facility, Microarrayer Facility.|

AB- <PB> PUBLICATIONS: Newsletter; Workshop brochures; LSC Booklet. EDUCATIONAL ACTIVITIES: Summer symposium (annually), in molecular biology; LSC Colloquium; Plant Physiology International Symposium; Nucleic acid and protein analysis workshop; Biological computing workshops; GCG (Genetic Computer Group) Workshop; Biomolecular Transport Dynamics Workshop. SCHOLARSHIPS: Life Science Consortium Fellowships, 40 per year. LIBRARY: Biotechnology Reading Room.|

SH- <Section> Biological and Environmental Sciences|

SH- <Number> 02|

DE- Biotechnology|Molecular biology|Botanical chemistry|Plant development||

3/4/46 (Item 2 from file: 115)

FN- DIALOG(R)File 115:Research Centers & Services|

CZ- (c) 2000 Gale Research Inc. All rts. reserv.|

AN- <Accession Nbr> 09987451|

AN- <Source Book> Research Centers Directory|RCD|

AN- <Internal No> 1640005720|

ON- <Org. Name> Harvard University|Center for Genomics Research (CGR)|

AD- <Street> 16 Divinity Ave.|

AD- <Last> Cambridge, MA 02138 USA|

OF- Dr. Dari Shalon, Dir.!

TE- <Phone> (617)495-8779|  
 FX- (617)495-2196|  
 EL- dari\_shalon@harvard.edu|  
 UR- http://www.cgr.harvard.edu|  
 AB- <OC> FOUNDED: 1999. DESCRIPTION: Integral unit of Harvard University.|  
 AB- <RD> RESEARCH: Genomics and proteomics. Some areas of research  
 undertaken by the multidisciplinary staff are bioinformatics,  
 microarray technology, protein analysis, biological imaging,  
 evolutionary analysis, genome manipulation, etc. RESOURCES: Q-Bot,  
 microarrayers gene chip systems.|  
 CT- <PS> INFO. OFFICER: Sylvie Agudelo.|  
 SH- <Section> Multidisciplinary Programs|  
 SH- <Number> 16|  
 DE- Biology|Medical genetics|Genomes||

3/4/47 (Item 1 from file: 133)

DIALOG(R) File 133:S&P's Corp.Descrip.+News

(c) 2001 McGraw-Hill Co. Inc. All rts. reserv.

00038514

Gene Logic Inc.

#### CORPORATE BACKGROUND

##### BUSINESS DESCRIPTION-

Gene Logic Inc. (GLGC) is an emerging participant in the burgeoning field of genomics. GLGC is building and commercializing a database based on gene expression -- the degree to which a gene is active in tissues in the body -- to identify genetic or protein targets for the development and marketing of potential pharmaceutical or biotechnology therapeutic candidates.

Scientists have long known that states of normal health and disease can be differentiated by specific genes and the proteins these genes direct cells to produce. By identifying how and to what degree there are differences in healthy and diseased specimens, researchers are able to identify specific sequences of genetic material that may be modified or manipulated to treat the disease. It is also possible to identify proteins that are controlled by specific genes, and to alter or supplement the body's ability to produce these proteins, thereby returning it to a healthy state.

GLGC generates gene expression data in its laboratories using two complementary technologies - GeneChip microarrays produced by Affymetrix, and proprietary patented READS technology. At year-end 2000, the GeneExpress Suite of databases contained gene expression profiles on over 4,000 tissue samples. By the end of 2003, GLGC expects to have complete gene expression profiles on 30,000 tissue samples, over half of which will be tissue from a wide range of human organs, each with comprehensive clinical information, representing a broad and in-depth survey of human gene expression across every major disease.

The company develops and licenses the GeneExpress software suite for the management and analysis of its growing database. The first product suite was released in November 1999. A second release in April 2000, GeneExpress 2000, incorporated enhanced analytical tools and an improved user interface. In addition, the company has been developing customized databases tailored to specific customer needs since 1997.

As of February 2001, Gene Logic had 15 clients for the GeneExpress database as well as numerous clients for its custom databases. Customers include Aventis CropScience, Fujisawa Pharmaceutical, Japan Tobacco, Merck & Co., NeuralStem, Organon, PE Biosystems, Pfizer, Procter & Gamble, Schering-Plough Research Institute, SmithKline Beecham, Therapeutic Genomics, UCB Pharma and Wyeth-Ayerst.

RESEARCH & DEVELOPMENT EXPENDITURES, Yrs. End. : Thous. \$

2000.....44,014 1999.....29,570 1998.....16,605

EMPLOYEES- December 31, 2000, 226.

CAPITAL EXPENDITURES, Thous. \$

2000.....9,685 1999.....3,229 1998.....6,873



SUBSIDIARIES-

Gene Logic Acquisition Corp.

INCORPORATED in Delaware Sept. 22, 1994 as Senatics Corp. Present title subsequently adopted.

OFFICERS-

Mark D. Gessler, President & Chief Executive Officer  
Phillip L. Rohrer, Chief Financial Officer  
Victor M. Markowitz, Sr V-P & Chief Informatin Officer  
J. B. Buzogany, General Counsel, Corp Secy & Sr. V-P  
Eric M. Eastman, Chief Technical Officer & Sr V-P  
Douglas Dolginow, M.D., Sr V-P  
David S. Murray, Sr V-P

DIRECTORS-

Jules Blake  
Micheal J. Brennan  
Charles L. Dimmler  
Mark D. Gessler  
G. Anthony Gorry  
Jeffery D. Sollender

OFFICE- 708 Quince Orchard Rd., Gaithersburg, MD 20878 (Tel.: 301-987-1700). Fax - 301-987-1701. WEBSITE-http://www.genelogic.com.

3/4/48 (Item 2 from file: 133)  
DIALOG(R)File 133:S&P's Corp.Descrip.+News  
(c) 2001 McGraw-Hill Co. Inc. All rts. reserv.

00029039

Incyte Genomics Inc.

CORPORATE BACKGROUND

BUSINESS DESCRIPTION-

Incyte Genomics, Inc. (INCY), formerly Incyte Pharmaceuticals, Inc., provides genomic information-based products and services. These products and services include database products, genomic data management software tools, **microarray** -based gene expression services, genomic reagents and related services. INCY focuses on providing an integrated platform of information technologies designed to assist pharmaceutical and biotechnology companies and academic researchers in the understanding of disease and the discovery and development of new drugs.

INCY's genomic databases integrate bioinformatics software with proprietary and, when appropriate, publicly available genomic information. In building the databases, INCY utilizes high-throughput, computer-aided gene sequencing and analysis technologies to identify and characterize the expressed genes of the human genome, as well as certain animal, plant and microbial genomes. By searching INCY's proprietary genomic databases, customers can integrate and analyze genomic information from multiple sources in order to discover genes that may represent the basis for new biological targets, therapeutic **proteins**, or gene therapy, antisense or diagnostic products. The pharmaceutical and biotechnology industries use INCY's genomic products and services to accelerate the discovery and development of new diagnostic and therapeutic products. INCY's products and services can be applied to gene and target discovery, functional genomics studies, preclinical pharmacology and toxicology studies, and can aid in understanding and analyzing the results of clinical development studies.

INCY provides access to its genomic databases through collaborations with pharmaceutical and biotechnology companies worldwide. As of Dec. 31, 1999, more than twenty companies had entered into multi-year agreements to obtain access to the company's databases on a non-exclusive basis.

RESEARCH & DEVELOPMENT EXPENDITURES, Yrs. End. Dec. 31.: Thous. \$  
1999.....146,833 1998.....97,192 1997.....68,900

EMPLOYEES- December 31, 1999, 1,108.

CAPITAL EXPENDITURES, Yrs. End. Dec. 31.: Thous. \$  
1999.....34,758 1998.....30,710 1997.....26,100

SUBSIDIARIES-

Incyte Europe Holdings Limited  
Incyte Europe Limited  
Synteni, Inc.

INCORPORATED in Delaware in April 1991 as Incyte Pharmaceuticals, Inc.  
Present title adopted June 15, 2000.

OFFICERS-

J. J. Collinson, Chairman  
R. A. Whitfield, President & Chief Exec Officer  
John M. Vuko, Chief Financial Officer  
R. W. Scott, Exec V-P & Chief Scientific Officer  
Richard Cathcart, V-P  
S. C. Clarke, V-P  
J. J. Seilhamer, V-P

DIRECTORS-

B. M. Bloom  
J. J. Collinson  
F. B. Craves  
J. S. Saxe  
R. W. Scott  
R. A. Whitfield

OFFICE- 3174 Porter Dr., Palo Alto, CA 94304 (Tel.: 650-855-0555). Fax -  
650-855-0572. WEBSITE-http://www.incyte.com.

3/4/99 (Item 1 from file: 144)

FN- DIALOG(R)File 144:Pascal|  
CZ- (c) 2001 INIST/CNRS. All rts. reserv.|  
AN- <DIALOG> 14795686|  
AN- <PASCAL No.> 00-0475982|  
TI- <English> Automated analysis of multivariate nonlinear gene relations  
based on cDNA microarray expression data|  
TI- <English> Advances in nucleic acid and protein analyses,  
manipulation, and sequencing : San Jose CA, 26-27 January 2000|  
AU- SEUNGCHAN KIM; DOUGHERTY E R; BITTNER M L; YIDONG CHEN; SIVAKUMAR K;  
MELTZER P; TRENT J M|  
AU- LIMBACH Patrick A, ed; OWICKI John C, ed; RAGHAVACHARI Ramesh, ed;  
WEIHONG TAN, ed|  
CS- Department of Electrical Engineering, Texas A&M University, United  
States; National Human Genome Research Institute, National Institutes  
of Health, United States; Department of Electrical Engineering,  
Washington State University, United States|  
CS- International Society for Optical Engineering, Bellingham WA, United  
States|  
CT- Advances in nucleic acid and protein analyses, manipulation, and  
sequencing. Conference|  
CL- San Jose CA USA|  
CY- 2000-01-26|  
JN- SPIE proceedings series|  
PY- 2000|  
VO- 3926|  
PG- 150-155|  
BN- 0-8194-3542-2|  
SN- 1017-2653|  
AV- INIST-21760; 354000090080960190|  
RF- 18 ref.|  
DT- P (Serial); C (Conference Proceedings)|  
DT- A (Analytic)|  
CP- United States|  
LA- English|  
AB- A cDNA microarray is a complex biochemical-optical system whose purpose  
is the simultaneous measurement of gene expression for thousands of  
genes. This paper describes a general statistical environment for

finding associations among gene expression patterns, and between genes and external conditions, via the coefficient of determination. This coefficient measures the degree to which the transcriptional levels of an observed gene set can be used to improve the prediction of the transcriptional state of a target gene relative to the best possible prediction in the absence of observations. Various aspects of the method are discussed: prediction quantification, design of predictors given small numbers of replicated microarrays, and constrained prediction using ternary perceptrons. A main focus is the supporting software and its facilities for data analysis and visualization.

DE- <English> Analysis method; Gene expression; Gene; Computer science; Software; Multivariate analysis; Non linear filter|  
DE- <French> Methode analyse; Expression genique; Gene; Informatique; Logiciel; Analyse multivariable; Filtre non lineaire; Reseau haute densite DNA complementaire|  
DE- <Spanish> Metodo analisis; Expresion genetica; Gen; Informatica; Logicial; Analisis multivariable; Filtro no lineal|  
SC- 002A04B|  
CR- Copyright (c) 2000 INIST-CNRS. All rights reserved.||

3/4/50 (Item 2 from file: 144)

FN- DIALOG(R)File 144:Pascal|  
CZ- (c) 2001 INIST/CNRS. All rts. reserv.|  
AN- <DIALOG> 14794508|  
AN- <PASCAL No.> 00-0474731|  
TI- <English> Expression ratio statistics and its applications to microarray data analysis|  
TI- <English> Advances in nucleic acid and protein analyses, manipulation, and sequencing : San Jose CA, 26-27 January 2000|  
AU- YIDONG CHEN; KAMAT V; DOUGHERTY E R; BITTNER M L; MELTZER P S; TRENT J M|  
AU- LIMBACH Patrick A, ed; OWICKI John C, ed; RAGHAVACHARI Ramesh, ed; WEIHONG TAN, ed|  
CS- Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, United States; Computer Assisted Medical Diagnostic Imaging Laboratory, Department of Electrical Engineering, Texas A&M University, College Station, Texas, United States|  
CS- International Society for Optical Engineering, Bellingham WA, United States|  
CT- Advances in nucleic acid and protein analyses, manipulation, and sequencing. Conference|  
CL- San Jose CA USA|  
CY- 2000-01-26|  
JN- SPIE proceedings series|  
PY- 2000|  
VO- 3926|  
PG- 142-149|  
BN- 0-8194-3542-2|  
SN- 1017-2653|  
AV- INIST-21760; 354000090080960180|  
RF- 4 ref.|  
DT- P (Serial); C (Conference Proceedings)|  
DT- A (Analytic)|  
CP- United States|  
LA- English|  
AB- Microarray technology makes it possible to monitor expression levels of thousands of genes simultaneously during single or multiple experiments. Routinely, in order to analyze gene expressions level quantitatively, two fluorescent-labeled RNAs are hybridized to an array of cDNA probes on a glass slide. Ratios of gene expression levels arising from two co-hybridized samples are obtained through image segmentation and signal detection methods. During the past three years, we have developed a gene expression analysis system in which ratio statistics have been applied to expression analysis, and a ratio confidence interval has been established to identify ratio outliers. By using local background subtraction and weak target elimination, we have been able to assume that the fluorescent background level does not

interfere with ratio measurement; however, experience suggests that ratios derived from either weak targets or in regions of high local background possess greater variation than those from strong targets. This paper proposes a new interaction model between fluorescent background and hybridization signals in which ratio statistics are numerically evaluated and a self-adjusting confidence interval is employed. The self-adjusting confidence interval, which automatically adapts under different signal-to-background ratios, provides a better criterion to further interrogate weak expression levels.

DE- <English> Analysis method; Gene expression; Gene; Statistical analysis; Ratio|

DE- <French> Methode analyse; Expression genique; Gene; Analyse statistique ; Ratio; Reseau haute densite DNA|

DE- <Spanish> Metodo analisis; Expresion genetica; Gen; Analisis estadistico; Ratio|

SC- 002A04B|

CR- Copyright (c) 2000 INIST-CNRS. All rights reserved.||

3/4/51 (Item 1 from file: 151)

FN- DIALOG(R)File 151:HealthSTAR|

CZ- (c) format only 2000 The Dialog Corporation. All rts. reserv.|

AN- 03713188|

AN- <NLM> 20433278|

TI- Printing proteins as microarrays for high-throughput function determination [see comments]|

AU- MacBeath G; Schreiber SL|

CS- Center for Genomics Research, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA. gavin macbeath@harvard.edu|

JN- Science; 289 (5485) p1760-3|

CP- UNITED STATES|

PY- Sep 8 2000|

SN- 0036-8075|

JC- UJ7|

NT- Comment in Science 2000 Sep 8;289(5485):1673|

LA- ENGLISH|

DT- JOURNAL ARTICLE|

JA- 0011|

SF- INDEX MEDICUS|

SF- MED/20433278|

AB- Systematic efforts are currently under way to construct defined sets of cloned genes for high-throughput expression and purification of recombinant proteins. To facilitate subsequent studies of protein function, we have developed miniaturized assays that accommodate extremely low sample volumes and enable the rapid, simultaneous processing of thousands of proteins. A high-precision robot designed to manufacture complementary DNA microarrays was used to spot proteins onto chemically derivatized glass slides at extremely high spatial densities. The proteins attached covalently to the slide surface yet retained their ability to interact specifically with other proteins, or with small molecules, in solution. Three applications for protein microarrays were demonstrated: screening for protein-protein interactions, identifying the substrates of protein kinases, and identifying the protein targets of small molecules.|

DE- \*Biochemistry --Methods --MT; \*Molecular Probe Techniques; \*Protein Binding; \*Protein Kinases --Metabolism --ME;

\*Proteins --Chemistry --CH \*Proteins --Metabolism --ME;

Biotin --Metabolism --ME; Digoxigenin --Metabolism --ME; Fluorescence ; Fluorescent Dyes; Ligands; Phosphorylation;

Piperazines --Pharmacology --PD; Protein Folding; Robotics; Serum Albumin, Bovine; Support, Non-U.S. Gov't|

RN- 0 (AF 1497); 0 (Fluorescent Dyes); 0 (Ligands); 0 (Piperazines) ; 0 (Proteins); 0 (Serum Albumin, Bovine); 1672-46-4 (Digoxigenin); 58-85-5 (Biotin)|

ID- EC 2.7.1.37 (Protein Kinases)|

3/4/52 (Item 1 from file: 155)

FN- DIALOG(R)File 155:MEDLINE(R)|

CZ- (c) format only 2000 Dialog Corporation. All rts. reserv. |  
 AN- 10591540 |  
 AN- <NLM> 20473216 |  
 TI- Picture story. A protein microarray . |  
 AU- Feng HP |  
 JN- Nature structural biology; 7 (10) p829 |  
 CP- UNITED STATES |  
 PY- Oct 2000 |  
 SN- 1072-8368 |  
 JC- B98 |  
 LA- ENGLISH |  
 DT- Journal Article |  
 SF- INDEX MEDICUS |  
 GS- Human |  
 DE- \*Proteins --chemistry --CH;  
 DE- Genome, Human |  
 RN- 0 (Proteins) |

3/4/53 (Item 1 from file: 165)

FN- DIALOG(R) File 165:EventLine(TM) |  
 CZ- (c) 2001 Elsevier Science B.V. All rts. reserv. |  
 AN- 00495021 |  
 TI- The World's First Meeting on Protein Microarray Technology |  
 ET- Conference |  
 DA- March 21-23, 2001 |  
 EY- 2001 |  
 ES- March 21-23, 2001 |  
 EE- March 21-23, 2001 |  
 HS- Hilton SD Resort  
 CY- San Diego  
 ST- California, USA  
 CN- USA |  
 RG- North America |  
 EX- No |  
 ON- IBC USA Conferences Inc. |  
 DP- Suite 400A |  
 AD- One Research Drive, POB 5195 |  
 CT- Westborough, MA 01581-5195 |  
 OC- USA |  
 TE- (508) 616-5550 |  
 FX- (508) 616-5522 |  
 SI- No |  
 DE- Pharmaceutics/Pharmacology 15000; Pharmaceutics - General 15010;  
 Biotechnology 18000; Biotechnology - General 18010; Biology 19000;  
 Genetics 19030; Immunology 19040; Microbiology 19050; Chemicals 46000;  
 Drugs and Pharmaceuticals 46040 |  
 PD- 010212 |  
 UR- ibcusa.com/2541 ||  
 EL- reg@ibcusa.com ||

3/4/54 (Item 1 from file: 174)

FN- DIALOG(R) File 174:Pharm-line(R) |  
 CZ- (c) CROWN COPYRIGHT 2001. All rts. reserv. |  
 AZ- 00174645 |  
 TI- AMPHOTERICIN B-INDUCED DIFFERENTIAL EXPRESSION OF GENES ENCODING  
 IMMUNOMODULATORY PROTEINS IN HUMAN T- AND B-LYMPHOCYTES DETECTED BY  
 CDNA MICROARRAY |  
 AU- Rogers PD et al. |  
 SO- Pharmacotherapy Oct 2000;20(10):1262 |  
 PY- 2000 |  
 AB- Paper presented at the 2000 Annual Meeting of the American College of  
 Clinical Pharmacy, Los Angeles, California, 5-8 Nov 2000. Abstract No.  
 204. In vitro study. Cell culture. |  
 DE- AMPHOTERICIN; GENES; T-LYMPHOCYTES; B-LYMPHOCYTES; IN VITRO TESTS; DNA;  
 RNA; PHARMACOGENETICS |

3/4/55 (Item 1 from file: 286)

FN- DIALOG(R)File 286:Biocommerce Abs.& Dir.|  
CZ- (c) 2001 BioCommerce Data Ltd. All rts. reserv.|  
AN- 01054385|  
CO- 57666 USE FORMAT 9 TO SEE COMPANY DIRECTORY RECORD|

3/4/56 (Item 2 from file: 286)

FN- DIALOG(R)File 286:Biocommerce Abs.& Dir.|  
CZ- (c) 2001 BioCommerce Data Ltd. All rts. reserv.|  
AN- 01054177|  
ST- NJ|  
CO- 48850 USE FORMAT 9 TO SEE COMPANY DIRECTORY RECORD|

3/4/57 (Item 3 from file: 286)

FN- DIALOG(R)File 286:Biocommerce Abs.& Dir.|  
CZ- (c) 2001 BioCommerce Data Ltd. All rts. reserv.|  
AN- 01045090|  
ST- Cambs|  
CO- 45490 USE FORMAT 9 TO SEE COMPANY DIRECTORY RECORD|

3/4/58 (Item 4 from file: 286)

FN- DIALOG(R)File 286:Biocommerce Abs.& Dir.|  
CZ- (c) 2001 BioCommerce Data Ltd. All rts. reserv.|  
AN- 00189693|  
JA- 20000927|  
DT- 1|  
ST- Revised|  
JN- Oxford GlycoSciences Press Release, 12SEP2000|  
JN- CAT Press Release, 12SEP2000|  
JN- BioWorld International, 20SEP2000, Vol(No) 5(39), Page(s) 1,2|  
AB- Oxford GlycoSciences (OGS) and Cambridge Antibody Technology (CAT) are  
to co-develop a **protein** chip technology for the detection of  
**proteins** using antibody based **microarrays**. Each company will fund  
its own research.|  
CO- Oxford GlycoSciences plc (OGS), UK (37757)|  
CO- Cambridge Antibody Technology Group plc (CAT), UK (41897)|

3/4/59 (Item 5 from file: 286)

FN- DIALOG(R)File 286:Biocommerce Abs.& Dir.|  
CZ- (c) 2001 BioCommerce Data Ltd. All rts. reserv.|  
AN- 00182091|  
JA- 19990729|  
DT- 7|  
ST- Revised|  
JN- Genetic Engineering News, JUL1999, Vol(No) 19(13), Page(s) 1,17,54|  
JN- BioVenture View, AUG1999, Vol(No) 14(7/8), Page(s) 16-17|  
JN- Nature Biotechnology, AUG1999, Vol(No) 17(8), Page(s) 741|  
AB- Reviews of Celltech's merger with Chiroscience which will create  
Europe's largest biotechnology company with a market capitalisation of  
over \$1 billion.|  
CO- Celltech plc, UK (42465)|  
CO- Chiroscience Group plc, UK (31909)|  
CO- Abbott Laboratories Inc, USA (38)|  
CO- **Peptide** Therapeutics Group plc, UK (33374)|  
CO- Proteus International plc, UK (1032)|  
CO- OraVax Inc, USA (24764)|  
CO- Therapeutic Antibodies Inc (TAb), USA (22885)|  
CO- Valentis Inc, USA (51189)|  
CO- British Biotech plc, UK (32923)|  
CO- Pharmacia & Upjohn Inc, USA (36197)|  
CO- SUGEN Inc, USA (25127)|  
CO- AstraZeneca plc, UK (47999)|  
CO- PolyMASC Pharmaceuticals plc, UK (37200)|  
CO- Novartis AG, Switzerland (38000)|  
CO- Millennium Pharmaceuticals Inc, USA (29628)|

CO- Molecular Dynamics Inc, USA ( 7431) |  
CO- Amersham Pharmacia Biotech AB, Sweden (44015) |  
CO- Synteni Inc, USA (36779) |  
CO- Affymetrix Inc, USA (26507) |  
CO- Incyte Microarray Systems, USA (54555) |  
CO- Celltech Chiroscience plc, UK (55011) |  
CO- Pharmacia Corp, USA (56022) |  
CO- Protherics plc, UK (55020) |  
CO- Protherics Inc, USA (56784) |  
CO- Celltech Group plc, UK (16049) |  
CO- Acambis plc, UK (57790) |  
CO- Acambis Inc, USA (57235) |

3/4/60 (Item 6 from file: 286)

FN- DIALOG(R) File 286: Biocommerce Abs. & Dir. |  
CZ- (c) 2001 BioCommerce Data Ltd. All rts. reserv. |  
AN- 00181900 |  
JA- 19990729 |  
DT- 7 |  
ST- Revised |  
JN- Nature Biotechnology Supplement, JUN1999, Vol(No) 17(6), Page(s) BE6-BE7 |  
AB- Review discussing factors to be considered when making a biotechnology company acquisition or disposal. |  
CO- PricewaterhouseCoopers (PwC), UK (47115) |  
CO- Medea International Healthcare Consulting, UK (18272) |  
CO- Phytera Inc, USA (30893) |  
CO- Auda Pharmaceuticals ApS, Denmark (42101) |  
CO- Fuisz Technologies Ltd, USA (25649) |  
CO- Trinity Biotech plc, Ireland (26737) |  
CO- Clark Laboratories Inc, USA ( 7522) |  
CO- Synteni Inc, USA (36779) |  
CO- Celltech Biologics plc, UK (29032) |  
CO- Alanex Corp, USA (27939) |  
CO- Agouron Pharmaceuticals Inc, USA (13285) |  
CO- Lonza AG, Switzerland ( 9200) |  
CO- Athena Neurosciences Inc, USA (12500) |  
CO- Elan Corp plc, Ireland ( 3654) |  
CO- Cambridge Antibody Technology Group plc (CAT), UK (41897) |  
CO- Aptein Inc, USA (25030) |  
CO- North American Vaccine Inc (NAVA), USA (22429) |  
CO- Cephalon Inc, USA (17773) |  
CO- Bayer AG, Germany ( 159) |  
CO- ALZA Corp, USA ( 1171) |  
CO- Monsanto Co, USA ( 1092) |  
CO- Watson Pharmaceuticals Inc, USA (30541) |  
CO- Chiron Diagnostics (US), USA (37594) |  
CO- Theratechnologies Inc, Canada (31122) |  
CO- TheraTech Inc, USA (12820) |  
CO- Peptide Therapeutics Group plc, UK (33374) |  
CO- SEQUUS Pharmaceuticals Inc, USA (35600) |  
CO- Ligand Pharmaceuticals Inc, USA (23397) |  
CO- DEKALB Genetics Corp, USA ( 9217) |  
CO- Virus Research Institute Inc, USA (28388) |  
CO- OncorMed Inc, USA (31250) |  
CO- Gene Logic Inc, USA (37419) |  
CO- AVANT Immunotherapeutics Inc, USA (45666) |  
CO- Seragen Inc, USA ( 2790) |  
CO- Hexagen plc, UK (39758) |  
CO- Incyte Microarray Systems, USA (54555) |  
CO- Elan Pharmaceuticals, USA (55226) |  
CO- Acambis plc, UK (57790) |

3/4/61 (Item 7 from file: 286)

FN- DIALOG(R) File 286: Biocommerce Abs. & Dir. |  
CZ- (c) 2001 BioCommerce Data Ltd. All rts. reserv. |  
AN- 00161674 |

JA- 19971006|  
DT- 2|  
ST- Revised|  
JN- Genetic Technology News, 10SEP1997, Vol(No) 17(17), Page(s) 2|  
AB- Researchers at the University of Texas Southwestern Medical Center are using *Saccharomyces cerevisiae* as a model to investigate disease gene interactions. Segments of genes will be displayed on a **microarray** chip to detect which RNAs are active in the yeast cells and the presence of an RNA will indicate that the disease causing **protein** is being produced.|  
CO- Texas, University of, Southwestern Medical Center, USA (15885)|

3/4/62 (Item 1 from file: 342)  
FN- DIALOG(R)File 342:Derwent Patents Citation Indx|  
CZ- (c) 2001 Derwent Info Ltd. All rts. reserv.|  
FN- World Patents Index (DIALOG File 342)|  
AX- 00-628276/60|  
TI- Identifying clones encoding membranal or secreted proteins for identifying targets of drug development, by deriving probes from membrane-bound and free polysomes and performing microarray-based comparison - |  
PA- (QUAR-) QUARK BIOTECH INC; (KOHN/) KOHN K I|  
AU- <INVENTORS> SKALITER R; EINAT P; MOR O; NOVAK L|  
NC- 090  
NT- 001  
NR- 004  
NG- 000  
PN- <BASIC> WO 200056935 A1 000928 ^(BASIC) |  
DW- <BASIC> 0060|  
PN- <EQUIVALENTS> AU 200039168 A 001009|  
AN- <PRIORITIES> US 125975 (990324)|  
AN- <APPLICATIONS> AU 200039168 (000324); WO 2000US7830 (000324)|  
DS- <NATIONAL> AE; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN; CR; CU; CZ; DE; DK; DM; EE; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; US; UZ; VN; YU; ZA; ZW  
DS- <REGIONAL> AT; BE; CH; CY; DE; DK; EA; ES; FI; FR; GB; GH; GM; GR; IE; IT; KE; LS; LU; MC; MW; NL; OA; PT; SD; SE; SL; SZ; TZ; UG; ZW  
IC- C12M-003/00|  
DC- B04; D16|  
CT- WO 200056935A US 6013437 A 98-297927/26 (QBIQ-) QBI ENTERPRISES LTD, (KOHN/) KOHN K I; LURIA S, EINAT P, HARRIS N, SKALITER R, GROSMAN Z|  
RF- \$  
RF- WO 200056935A MECHLER, M. M.: 'Isolation of Messenger RNA from Membrane Bound Polysomes' METHODS IN ENZYMOLOGY vol. 152, 1987, pages 241 - 248, XP002928698  
RF- WO 200056935A BHARUCHA ET. AL.: 'Characterization of polysomes and polysomal mRNAs by sucrose density gradient centrifugation followed by immobilization in polyacrylamide gel matrix' METHODS IN ENZYMOLOGY vol. 216, 1992, pages 168 - 179, XP002928699  
RF- WO 200056935A SCHENA ET. AL.: 'Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes' PROC. NATL. ACAD. SCI. USA. vol. 93, October 1996, pages 10614 - 10619, XP002928700  
RF- WO 200056935A CHEE ET. AL.: 'Accessing Genetic Information with High-Density DNA arrays' SCIENCE vol. 274, 25 October 1996, pages 610 - 614, XP002022508|

3/4/63 (Item 1 from file: 351)  
DIALOG(R)File 351:Derwent WPI  
(c) 2001 Derwent Info Ltd. All rts. reserv.

AA- 2001-201999/200120|  
XR- <XRAM> C01-059917|  
TI- Composition having probes which comprise part of gene sequence encoding proteins associated with cell proliferation useful as hybridizable



array elements in Microarrays to monitor expression of target polynucleotide|

PA- INCYTE PHARM INC (INCY-N)|

AU- <INVENTORS> BANDMAN O; BAUGHN M R; GUEGLER K J; HILLMAN J L; LAL P; REDDY R; YUE H|

NC- 001|

NP- 001|

PN- US 6183968 B1 20010206 US 9879677 A 19980327 200120 B

<AN> US 99276531 A 19990325|

AN- <LOCAL> US 9879677 A 19980327; US 99276531 A 19990325|

AN- <PR> US 9879677 A 19980327; US 99276531 A 19990325|

FD- US 6183968 B1 C12Q-001/68 Provisional application US 9879677|

LA- US 6183968(104)|

AB- <PN> US 6183968 B1|

AB- <NV> NOVELTY - A composition (I) comprising several polynucleotide probes (II), is new.|

AB- <BASIC> DETAILED DESCRIPTION - 134 (S1-S134) probe sequences are claimed in the specification such as probes having a fully defined sequence of 1419 (S1), 468 (S2), 2351 (S3), 887 (S4), 1350 (S5), 102 (S6), 1233 (S7), 3556 (S8) base pairs as given in the specification.

USE - (II) is immobilized and is preferably useful as hybridizable array elements in a microarray (claimed) for monitoring the expression of several polynucleotides. The microarray can be used in the diagnosis of cancers such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma and tetratocarcinoma etc., immunopathology such as AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis and bronchitis etc., neuropathology such as Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia and cerebral neoplasms etc. The microarray can be used for large scale genetic or gene expression analysis of a large number of target polynucleotides. The microarray can also be used in the diagnosis of diseases and in the monitoring of treatments where altered expression of genes coding for the proteins associated with cell proliferation or receptors cause disease. The microarray can also be used to investigate an individual's predisposition to a disease such as cancer, immunopathology or neuropathology. Also, they can be used for investigating cellular response to infection, drug treatment etc. The microarray can be used for diagnostics, prognostics and treatment regimens, drug discovery and development, toxicological and carcinogenicity studies, forensics, pharmacogenomics etc. The arrays can also be used for monitoring disease progression. (I) can be used to purify a subpopulation of mRNAs, cDNAs, genomic fragments etc., in a sample.

ADVANTAGE - When (I) is employed as hybridizable array elements in a microarray, the array elements are organized in an ordered fashion so that each element is present at a specified location on the substrate. Because the array elements are at specified locations on the substrate, the hybridization patterns and intensities (which together create a unique expression profile) can be interpreted in terms of expression levels of particular genes and can be correlated with a particular disease or condition or treatment.

pp; 104 DwgNo 0/0|

AB- <TF> TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Oligonucleotides: (II) comprises a nucleotide sequence coding for proteins associated with cell proliferation or for receptors. Preferably, each of (II) comprises (S1-S8), 774 (S9), 541 (S10), 250 (S11), 1563 (S12), 517 (S13), 1209 (S14), 556 (S15), 4245 (S16), 50 (S17), 1464 (S18), 1992 (S19), 796 (S20), 2101 (S21), 2138 (S22) base pairs as given in the specification. Preferably, (II) comprises a fully defined nucleotide sequence of (S22-S134) as given in the specification.|

DE- <TITLE TERMS> COMPOSITION; PROBE; COMPRISE; PART; GENE; SEQUENCE; ENCODE; PROTEIN; ASSOCIATE; CELL; PROLIFERATION; USEFUL; ARRAY; ELEMENT ; MONITOR; EXPRESS; TARGET; POLYNUCLEOTIDE|

DC- B04; D16|

IC- <MAIN> C12Q-001/68|

MC- <CPI> B04-E05; B11-C08E; B11-C08E5; B12-K04A; B12-K04E; B12-K04F; D05-H09; D05-H10; D05-H12D1|

FS- CPI||

3/4/64 (Item 2 from file: 351)

DIALOG(R) File 351: Derwent WPI

(c) 2001 Derwent Info Ltd. All rts. reserv.

IM- \*Image available\*

AA- 2001-025170/200103|

XR- <XRAM> C01-007783|

TI- Producing microarray chip of chemical compounds e.g. DNAs, peptide nucleic acids and polypeptides for use in biological assay, drug screening and gene sequence analysis, with high space resolution and accuracy, at low cost|

PA- LU Z (LUZZ-I)|

AU- <INVENTORS> HE N; LU Z; ZHAO Y; MA J|

NC- 091|

NP- 003|

PN- WO 200071746 A1 20001130 WO 2000CN125 A 20000519 200103 B|

PN- AU 200045354 A 20001212 AU 200045354 A 20000519 200115

PN- CN 1274758 A 20001129 CN 99106790 A 19990521 200121|

AN- <LOCAL> WO 2000CN125 A 20000519; AU 200045354 A 20000519; CN 99106790 A 19990521|

AN- <PR> CN 99106790 A 19990521|

FD- WO 200071746 A1 C12Q-001/68

<DS> (National): AE AL AM AT AU AZ BA BB BG BR BY CA CH CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

<DS> (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW

FD- AU 200045354 A C12Q-001/68 Based on patent WO 200071746|

LA- WO 200071746(C<PG> 23)|

DS- <NATIONAL> AE AL AM AT AU AZ BA BB BG BR BY CA CH CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW|

DS- <REGIONAL> AT; BE; CH; CY; DE; DK; EA; ES; FI; FR; GB; GH; GM; GR; IE; IT; KE; LS; LU; MC; MW; MZ; NL; OA; PT; SD; SE; SL; SZ; TZ; UG; ZW|

AB- <PN> WO 200071746 A1|

AB- <NV> NOVELTY - A method for producing a microarray chip of chemical compounds, is new.|

AB- <BASIC> DETAILED DESCRIPTION - A method for producing a microarray chip of chemical compounds comprises:

(a) designing a microarray chip of the chemicals according to requirements and preparing the specific micro reaction chamber;

(b) immobilizing the reaction chamber onto a substrate with a fixed point device to form a micro reaction chamber fixed point synthesis system;

(c) introducing a reaction solution to the synthesis system for chemical reaction on the substrate surface after contacting with the micro reaction chamber; and

(d) controlling the fixed point synthesis by changing the reaction chamber, or by regulating the flow of liquid in the reaction chamber to provide a microarray chip formed with various compounds on the substrate.

USE - The method is for producing microarray chip of chemical compounds, particularly DNA, peptide nucleic acid and polypeptide microarray chips (claimed) for use in biological assay, diagnosis, drug screening, gene sequence analysis and combinatorial synthesis.

ADVANTAGE - The chips are reliable, have high space resolution and accuracy, and are produced at a low cost.

DESCRIPTION OF DRAWING(S) - Structure of a micro reaction chamber. pp; 23 DwgNo 1/13|

AB- <TF> TECHNOLOGY FOCUS - INSTRUMENTATION AND TESTING - Preferred

Equipment: The micro reaction chamber is a patterned mold with protrusions and recesses designed which are obtained by micromachining e.g. on a silicon wafer substrate through photomasking and etching then adding monomers for polymerization and curing to give a molded plate. The patterning can be just on the silicon wafer surface, or directly machined through the wafer with a laser beam, particle beam, or

microknife tool. The reaction chamber can also be a combination of different molded plates and microfluid cover plates, optionally equipped with micro-valves and electrodes to control the liquid flow in the various chambers. A catalyst or bioenzyme can also be added in step (c) to promote the bonding of compound(s) onto the substrate. In step (d), the fixed point synthesis can be performed with overlapping or without on the same point of the reaction chamber on the substrate. The reaction can be generated through an equivalent energy from sound, light, heat, electricity or/and magnetism with control, which is particularly carried out under vacuum or in an inert gas.

DE- <TITLE TERMS> PRODUCE; CHIP; CHEMICAL; COMPOUND; PEPTIDE; NUCLEIC; ACID  
; BIOLOGICAL; ASSAY; DRUG; SCREEN; GENE; SEQUENCE; ANALYSE; HIGH; SPACE  
; RESOLUTION; ACCURACY; LOW; COST|

DC- B04; D16|

IC- <MAIN> C12Q-001/68|

IC- <ADDITIONAL> G01N-033/15|

MC- <CPI> B04-B03C; B04-C01; B04-E01; B04-E05; B11-C; B12-K04; D05-H09;  
D05-H10; D05-H12D; D05-H12D1|

FS- CPI||

3/4/65 (Item 3 from file: 351)

DIALOG(R) File 351:Derwent WPI

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IM- \*Image available\*

AA- 2000-672763/200065|

XR- <XRAM> C00-203826|

XR- <XRPX> N00-498763|

TI- Simultaneous detection of many protein-binding ligands, useful e.g.  
for diagnosis and drug screening, uses high-density microarray of  
immobilized peptides |

PA- UNIV LELAND STANFORD JUNIOR (STRD )|

AU- <INVENTORS> BROWN P; HAAB B|

NC- 021|

NP- 001|

PN- WO 200063701 A2 20001026 WO 2000US10171 A 20000414 200065 B|

AN- <LOCAL> WO 2000US10171 A 20000414|

AN- <PR> US 99129449 A 19990415|

FD- WO 200063701 A2 G01N-033/68

<DS> (National): CA JP US

<DS> (Regional): AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE|

LA- WO 200063701(E<PG> 33)|

DS- <NATIONAL> CA JP US|

DS- <REGIONAL> AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC;  
NL; PT; SE|

AB- <PN> WO 200063701 A2|

AB- <NV> NOVELTY - Simultaneous detection of many protein-binding ligands  
(I) in a sample comprises contacting the sample with a microarray of  
peptides (II), washing to remove unbound sample and detecting bound  
(I). The microarray has at least 100 discrete regions, carrying  
different (I), per cm<sup>2</sup> of solid planar support.|

AB- <BASIC> USE - The method is particularly used to detect and quantify  
antigens or antibodies, but may also be used to detect any  
protein-interacting compound, e.g. a polynucleotide, hormone, vitamin,  
co-factor etc., e.g. in large scale diagnostic assays (for detecting  
disease markers, or for assessing response to drugs or environmental  
factors); for drug development and generally in molecular biology,  
immunology and toxicology.

ADVANTAGE - The miniaturized microarray uses far less sample than  
traditional immunoassays and analysis of many (I) in parallel allows  
diagnosis to be made from a multidimensional pattern of expression.  
Where measurements are taken by comparative fluorescence, greater  
precision over a wide range of ligand concentrations and binding  
affinities is achieved, compared with measuring the absolute amount of  
bound ligand.

DESCRIPTION OF DRAWING(S) - The figure shows a side view of a  
reagent-dispensing device having an open-capillary dispensing head.

pp: 33 DwgNo 1/8

AB- <TF> TECHNOLOGY FOCUS - BIOLOGY - Preferred Materials: (I) in the sample are labeled, particularly with fluorophores. The sample may be a biological fluid (especially blood or its derivatives), a cell culture supernatant or a cell lysate. (II) are particularly antibodies or antigens and contain at least 50 amino acids.

Preferred Process: The sample and microarray are incubated for about 1 hr, then the array is washed (optionally several times) in pH 7-8 medium containing a nonionic detergent. Optionally the array is reacted with a second sample containing ligands that carry different fluorophore labels.

DE- <TITLE TERMS> SIMULTANEOUS; DETECT; PROTEIN; BIND; LIGAND; USEFUL; DIAGNOSE; DRUG; SCREEN; HIGH; DENSITY|

DC- B04; D16; S03|

IC- <MAIN> G01N-033/68|

MC- <CPI> B03-L; B04-B04C; B04-B04D; B04-C01; B04-C03; B04-G01; B04-J01; B11-C07A; B11-C07B3; B12-K04E; D05-H09; D05-H10; D05-H11; D05-H12D|

MC- <EPI> S03-E14H|

FS- CPI; EPI||

3/4/66 (Item 4 from file: 351)

DIALOG(R) File 351:Derwent WPI

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AA- 2000-628276/200060|

XR- <XRAM> C00-188263|

TI- Identifying clones encoding membranal or secreted proteins for identifying targets of drug development, by deriving probes from membrane-bound and free polysomes and performing microarray-based comparison|

PA- QUARK BIOTECH INC (QUAR-N); KOHN K I (KOHN-I)|

AU- <INVENTORS> EINAT P; MOR O; NOVAK L; SKALITER R|

NC- 090|

NP- 002|

PN- WO 200056935 A1 20000928 WO 2000US7830 A 20000324 200060 B|

PN- AU 200039168 A 20001009 AU 200039168 A 20000324 200103|

AN- <LOCAL> WO 2000US7830 A 20000324; AU 200039168 A 20000324|

AN- <PR> US 99125975 A 19990324|

FD- WO 200056935 A1 C12Q-001/68

<DS> (National): AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

<DS> (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

FD- AU 200039168 A C12Q-001/68 Based on patent WO 200056935|

LA- WO 200056935(E<PG> 29)|

DS- <NATIONAL> AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW|

DS- <REGIONAL> AT; BE; CH; CY; DE; DK; EA; ES; FI; FR; GB; GH; GM; GR; IE; IT; KE; LS; LU; MC; MW; NL; OA; PT; SD; SE; SL; SZ; TZ; UG; ZW|

AB- <PN> WO 200056935 A1|

AB- <NV> NOVELTY - A method for identifying clones which encode membranal or secreted proteins comprising preparing cDNA probes from total RNA extracted from membrane bound polysomes and free polysomes and microarray based comparison of the relative abundance of the different RNA species, is new.|

AB- <BASIC> USE - The method is useful for identifying clones encoding membranal or secreted proteins which provides an efficient tool for identifying targets of drug development. The method serves as a tool for augmenting conventional differential analysis of relative mRNA abundance in different RNA sources by virtue or its use of RNA extracted from specific subcellular compartments as templates for cDNA probes (claimed).

ADVANTAGE - The method allows rapid identification of highly relevant membranal and secreted proteins.

pp: 29 DwgNo 0/1

AB- <TF> TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: Total RNA is prepared by cell fractionation which includes creating polysomal subdivisions which can discriminate between total polyribosomes and membrane-bound-polysomes. The intact total RNA is then extracted from specific cellular compartments by sucrose step analysis. The method further includes repairing a cell lysate using TEA (not defined) based hypotonic buffer, ascertaining mRNA intactness using RNase inhibitors and separating rough endoplasmic reticulum membranes using sucrose step gradient.

DE- <TITLE TERMS> IDENTIFY; CLONE; ENCODE; SECRETION; PROTEIN; IDENTIFY; TARGET; DRUG; DEVELOP; DERIVATIVE; PROBE; MEMBRANE; BOUND; FREE; PERFORMANCE; BASED; COMPARE|

DC- B04; D16|

IC- <MAIN> C12Q-001/68|

IC- <ADDITIONAL> C12M-003/00|

MC- <CPI> B04-E05; B04-M01; B04-N04; B11-B; B11-C08E5; B12-K04E; B12-K04F; D05-H09; D05-H12D1|

FS- CPI||

3/4/67 (Item 5 from file: 351)  
 DIALOG(R) File 351:Derwent WPI  
 (c) 2001 Derwent Info Ltd. All rts. reserv.

AA- 2000-611434/200058|

XR- <XRAM> C00-182920|

TI- **Microarray of peptide** affinity probes useful for analyzing and quantitating gene products is formed on the porous membrane proximate to an electrode|

PA- COMBIMATRIX CORP (COMB-N)|

AU- <INVENTORS> MONTGOMERY D D; ROSSI F M|

NC- 089|

NP- 002|

PN- WO 200053625 A2 20000914 WO 2000US6676 A 20000310 200058 B|

PN- AU 200038833 A 20000928 AU 200038833 A 20000310 200067|

AN- <LOCAL> WO 2000US6676 A 20000310; AU 200038833 A 20000310|

AN- <PR> US 99123877 A 19990311; US 99123791 A 19990311|

FD- WO 200053625 A2 C07K-001/00

<DS> (National): AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZW

<DS> (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

FD- AU 200038833 A C07K-001/00 Based on patent WO 200053625|

LA- WO 200053625(E<PG> 52)|

DS- <NATIONAL> AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZW|

DS- <REGIONAL> AT; BE; CH; CY; DE; DK; EA; ES; FI; FR; GB; GH; GM; GR; IE; IT; KE; LS; LU; MC; MW; NL; OA; PT; SD; SE; SL; SZ; TZ; UG; ZW|

AB- <PN> WO 200053625 A2|

AB- <NV> NOVELTY - An array (I) of peptide probes comprising a porous membrane proximate to at least one electrode, is new.|

AB- <BASIC> DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) producing an array of peptide probes for analyzing gene products comprising:

(a) providing a substrate having at its surface at least one electrode that is proximate to an amino acid bearing a protected chemical functional group;

(b) applying a potential to the electrode sufficient to generate electrochemical reagents capable of deprotecting the protected chemical functional groups of the amino acid; and

(c) bonding the deprotected chemical functional group with another amino acid; and

(2) making an array of one or more peptide probes for analyzing gene products comprising:

(a) placing a buffering or scavenging solution in contact with an array of electrodes that is proximate to substrate surface, where the surface being proximate to one or more amino acids bearing a protected chemical functional group attached to it;

(b) selectively deprotecting a protected chemical functional group on the amino acids;

(c) bonding another amino acid having a protected chemical functional group to one or more deprotected chemical functional groups of the amino acid; and

(d) repeating the selective deprotection of a chemical functional group on a bonded protected amino acid and subsequently bonding an additional amino acid to the deprotected chemical functional group until at least two separate peptides of desired length are formed on the substrate surface.

USE - For determining the presence or quantitating the amount of a gene product in a biological sample. (I) is also useful for identifying the functional binding domain of a gene product in a sample (claimed). The peptide probe arrays are useful for discovering new drug targets.

ADVANTAGE - The gene products that are difficult to observe by two dimensional electrophoresis such as hydrophobic, basic and large proteins can be observed. Variability resulting from differences in sample preparation and differences in susceptibility of protein to stain is reduced. Peptide probes are placed on a array defined pattern which eliminates the need for complex pattern recognition software. The peptide array allows gene product expression levels, amino acid sequence and potential interaction between the gene product and other peptide to be determined. The method significantly reduces the time required to develop new drugs.

pp; 52 DwgNo 0/12|

AB- <TF> TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Array: (I) comprises at least 100, preferably 1000 electrodes and the electrode current or potential is controlled and operated by interface with a computer.

Preferred Method: The method involves preparing a peptide array on a porous substrate, contacting the sample with the peptide array, and observing binding of the gene product to the peptide probe array, where the gene product is labeled with a fluorescent label. Functional binding domains are identified by providing a spatially coded array of peptides, binding several antibodies comprising a known amino acid sequence to the peptides, contacting the array with a biological sample and monitoring binding of molecules in the biological sample to the antibodies.

DE- <TITLE TERMS> PEPTIDE; AFFINITY; PROBE; USEFUL; QUANTIFICATION; GENE; PRODUCT; FORMING; POROUS; MEMBRANE; PROXIMATE; ELECTRODE|

DC- B04; D16|

IC- <MAIN> C07K-001/00|

MC- <CPI> B04-E01; B04-N04; B11-C08; B12-K04; D05-H09|

FS- CPI||

3/4/68 (Item 1 from file: 357)

FN- DIALOG(R) File 357: Derwent Biotechnology Abs|

CZ- (c) 2001 Derwent Publ Ltd. All rts. reserv.|

AZ- 0261985|

AZ- 2001-00454|

TI- Microarray of peptide affinity probes useful for analyzing and quantiating gene products is formed on the porous membrane proximate to an electrode|

TI- peptide probe|

AU- Rossi F M Montgomery D D|

PA- Combimatrix|

PN- WO 200053625|

PD- 20000914|

CS- Burlingame, CA, USA.|

CS- Combimatrix|

SO- Combimatrix|

PY- 2000|

CD- Combimatrix|

LA- English|

AB- An array of peptide probes containing a porous membrane proximate to at

least one electrode, is new. Also claimed are: producing an array of peptide probes for analyzing gene products; and making an array of one or more peptide probes for analyzing gene products. The methods can be used for determining the presence or quantitating the amount of a gene product in a biological sample. The array is also useful for identifying the functional binding domain of a gene product in a sample. The peptide probes arrays are useful for discovering new drug targets. (52pp)|

SO- Burlingame, CA, USA.|

DE- gene product quantitation, peptide probe, electrode (Vol.20, No.2)|

SH- GENETIC ENGINEERING AND FERMENTATION Nucleic Acid Technology|

SC- A1|

3/4/69 (Item 1 from file: 399)

FN- DIALOG(R)File 399:CA SEARCH(R)|

CZ- (c) 2001 AMERICAN CHEMICAL SOCIETY. All rts. reserv. |

AZ- 134219193|

AZ- 134(16)219193j|

TI- Miniaturized electrospraying as a technique for the production of microarrays of reproducible micrometer sized protein spots|

DT- CONFERENCE PROCEEDING|

AU- Moerman, Robert; Frank, Johannes; Marijnissen, Johannes C. M.; Schalkhammer, Thomas; van Dedem, Gijs W. K. |

AU- <EDITOR> Van den Berg, Albert (Ed); Olthuis, W. (Ed); Bergveld, Piet (Ed); |

CS- <LOCATION> Kluyver Laboratory of Biotechnology; University of Technology; 2628 BC; Delft; Neth. |

PU- Kluwer Academic Publishers, Dordrecht, Neth|

JN- Micro Total Anal. Syst. 2000, Proc. .mu.TAS Symp., 4th, P557-560|

PY- 2000|

CO- 69AJPB|

LA- English|

SC- CA209007 Biochemical Methods|

ID- miniaturized electrospraying microarray protein|

DE- Electrodes

(counter; miniaturized electrospraying as a technique for prodn. of microarrays of reproducible micrometer sized protein spots)|

DE- Spraying

(electrospraying; miniaturized electrospraying as a technique for prodn. of microarrays of reproducible micrometer sized protein spots)|

DE- Capillary tubes; Drops; Electric field; Electrolytes; Meniscus; Proteins, general, processes; Reagents; Surface electric charge; Surfactants

(miniaturized electrospraying as a technique for prodn. of microarrays of reproducible micrometer sized protein spots)|

RN- 56-81-5 107-21-1

uses, miniaturized electrospraying as a technique for prodn. of microarrays of reproducible micrometer sized protein spots|

\*

3/4/70 (Item 2 from file: 399)

FN- DIALOG(R)File 399:CA SEARCH(R)|

CZ- (c) 2001 AMERICAN CHEMICAL SOCIETY. All rts. reserv. |

AZ- 134159600|

AZ- 134(12)159600c|

TI- Protein microarrays for monitoring of structural changes of proteins via surface enhanced metal nano cluster resonance|

DT- CONFERENCE PROCEEDING|

AU- Mayer, Christian; Palkovits, Roland; Bauer, Georg; Schalkhammer, Thomas |

AU- <EDITOR> Van den Berg, Albert (Ed); Olthuis, W. (Ed); Bergveld, Piet (Ed); |

CS- <LOCATION> Kluyver L. for Biotechnology; TU-Delft; 2628BC; Delft; Neth. |

PU- Kluwer Academic Publishers, Dordrecht, Neth|

JN- Micro Total Anal. Syst. 2000, Proc. .mu.TAS Symp., 4th, P553-556|

PY- 2000|

CO- 69AJPB|  
 LA- English|  
 SC- CA209001 Biochemical Methods; CA206XXX General Biochemistry|  
 ID- protein structure microarray surface enhanced resonance immobilization  
 crosslinking|  
 DE- Biosensors  
 (optical; protein microarrays for monitoring of structural changes of  
 proteins via surface enhanced metal nano cluster resonance)|  
 DE- Crosslinking; Immobilization; biochemical; Mirrors; pH; UV radiation  
 (protein microarrays for monitoring of structural changes of proteins  
 via surface enhanced metal nano cluster resonance)|  
 DE- Conformation  
 (protein; protein microarrays for monitoring of structural changes of  
 proteins via surface enhanced metal nano cluster resonance)|  
 DE- Optical absorption  
 (resonance; protein microarrays for monitoring of structural changes of  
 proteins via surface enhanced metal nano cluster resonance)|  
 DE- Albumins; properties  
 (serum; protein microarrays for monitoring of structural changes of  
 proteins via surface enhanced metal nano cluster resonance)|  
 RN- 50-01-1 2718-90-3 3179-76-8 9001-37-0 9002-13-5 25087-17-6  
 protein microarrays for monitoring of structural changes of  
 proteins via surface enhanced metal nano cluster resonance  
 RN- 7429-90-5 7440-06-4 7440-47-3 7440-57-5  
 uses, protein microarrays for monitoring of structural changes of  
 proteins via surface enhanced metal nano cluster resonance|  
 \*

3/4/71 (Item 3 from file: 399)

FN- DIALOG(R) File 399: CA SEARCH(R)|  
 CZ- (c) 2001 AMERICAN CHEMICAL SOCIETY. All rts. reserv.|  
 AZ- 133307185|  
 AZ- 133(22)307185t|  
 TI- Microarrays for the analysis of protein-ligand interactions|  
 DT- JOURNAL|  
 AU- Gamer, Jurgen|  
 CS- <LOCATION> Graffinity Pharmaceutical Design GmbH; Heidelberg; Germany;  
 |  
 PU- Spektrum Akademischer Verlag|  
 JN- BIOSpektrum, V6, N5, P415-416|  
 PY- 2000|  
 CO- BOSPFD|  
 SN- 0947-0867|  
 LA- German|  
 RP- 5|  
 SC- CA209002 Biochemical Methods|  
 ID- microarray protein ligand interaction|  
 DE- Proteins, specific or class  
 (immobilized; microarrays for the anal. of protein-ligand interactions  
 )|  
 DE- Proteins, specific or class  
 (ligand-binding; microarrays for the anal. of protein-ligand  
 interactions)|  
 DE- cDNA; Ligands  
 (microarrays for the anal. of protein-ligand interactions)|  
 \*

3/4/72 (Item 4 from file: 399)

FN- DIALOG(R) File 399: CA SEARCH(R)|  
 CZ- (c) 2001 AMERICAN CHEMICAL SOCIETY. All rts. reserv.|  
 AZ- 133293182|  
 AZ- 133(21)293182a|  
 TI- Preparation of and methods for use of polypeptide microarrays|  
 DT- PATENT|  
 AU- <INVENTOR> Brown, Patrick; Haab, Brian|  
 CS- <LOCATION> USA|  
 PA- The Board of Trustees of the Leland Stanford Junior University|



PN- PCT International ; WO 200063701 A2|  
 PD- 20001026|  
 AN- WO 2000US10171 (20000414); \*US PV129449 (19990415)|  
 JN- , P34 pp.|  
 CO- PIXXD2|  
 LA- English|  
 CL- G01N-033/68A|  
 DC- CA; JP; US|  
 DR- AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE|  
 SC- CA209001 Biochemical Methods|  
 ID- microarray biochip polypeptide ligand antibody immobilization diagnosis  
 |  
 DE- Biotechnology  
 (biochips; prepn. of and methods for use of polypeptide microarrays)|  
 DE- Dispensing apparatus  
 (dosing; prepn. of and methods for use of polypeptide microarrays)|  
 DE- Analytical apparatus; Antibodies; Antigens; Blood analysis; Body fluid;  
 Diagnosis; Fluorometry; Immobilization, biochemical; Immunoassay;  
 Ligands; Peptides, uses  
 (prepn. of and methods for use of polypeptide microarrays)|  
 \*

3/4/73 (Item 5 from file: 399)

FN- DIALOG(R) File 399:CA SEARCH(R)|  
 CZ- (c) 2001 AMERICAN CHEMICAL SOCIETY. All rts. reserv.|  
 AZ- 133219784|  
 AZ- 133(16)219784g|  
 TI- Microarrays of peptide affinity probes for analyzing patterns of  
 protein synthesis and their synthesis and methods for analyzing gene  
 products|  
 DT- PATENT|  
 AU- <INVENTOR> Rossi, Francis M.; Montgomery, Donald D.|  
 CS- <LOCATION> USA|  
 PA- Combimatrix Corporation|  
 PN- PCT International ; WO 200053625 A2|  
 PD- 20000914|  
 AN- WO 2000US6676 (20000310); \*US PV123877 (19990311); \*US PV123791  
 (19990311)|  
 JN- , P52 pp.|  
 CO- PIXXD2|  
 LA- English|  
 CL- C07K-001/00A|  
 DC- AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN; CR; CU; CZ; DE; DK;  
 DM; DZ; EE; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE;  
 KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX;  
 NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA;  
 UG; US; UZ; VN; YU; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM|  
 DR- GH; GM; KE; LS; MW; SD; SL; SZ; TZ; UG; ZW; AT; BE; CH; CY; DE; DK; ES;  
 FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA;  
 GN; GW; ML; MR; NE; SN; TD; TG|  
 SC- CA209002 Biochemical Methods; CA203XXX Biochemical Genetics; CA234XXX  
 Amino Acids, Peptides, and Proteins|  
 ID- peptide affinity probe proteome analysis bioelectrode|  
 DE- Fluorescent dyes  
 (as reporter groups; microarrays of peptide affinity probes for  
 analyzing patterns of protein synthesis and their synthesis and methods  
 for analyzing gene products)|  
 DE- Membrane electrodes  
 (bioelectrodes, peptide arrays immobilized on; microarrays of peptide  
 affinity probes for analyzing patterns of protein synthesis and their  
 synthesis and methods for analyzing gene products)|  
 DE- Protective groups  
 (Fmoc, in synthesis of peptides on membrane electrodes; microarrays of  
 peptide affinity probes for analyzing patterns of protein synthesis and  
 their synthesis and methods for analyzing gene products)|  
 DE- Electrochemistry  
 (for anal. of patterns of protein synthesis; microarrays of peptide  
 affinity probes for analyzing patterns of protein synthesis and their

synthesis and methods for analyzing gene products)|

DE- Semiconductor devices  
(for synthesis of peptide probe arrays; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products)|

DE- Antibodies  
(in anal. of patterns of gene expression and of gene product ligand binding domain; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods fo)|

DE- Peptide library  
(ordered arrays; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products)|

RN- 76197-35-8 92557-80-7  
as reporter group; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

RN- 68-19-9 1941-24-8  
in prepn. of peptide arrays; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

RN- 58822-25-6P  
synthesis on membrane electrode of; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products|

\*

3/4/74 (Item 1 from file: 420)  
DIALOG(R) File 420:UnCover  
(c) 2001 The UnCover Company. All rts. reserv.

AZ- 14072324|  
AA- 251130199197|  
TI- New Protein Microarrays . |  
JN- Chemical and engineering news|  
JC- 23182036|  
PD- September 11, 2000|  
VO- v. 78 n. 37|  
PG- 6|  
SN- 0009-2347|  
AB- Devices could ease screening of cellular proteins en masse.|

3/4/75 (Item 1 from file: 431)  
FN- DIALOG(R) File 431:MediConf: Medical Con. & Events|  
CZ- (c) 2001 Dr. R. Steck. All rts. reserv.|  
AZ- 00085975|  
AA- 18943901|  
TI- Protein Microarray Technology|  
ET- Seminar|  
ES- from Wednesday, March 21, 2001|  
EE- to Wednesday, March 21, 2001|  
HS- The Westin Horton Plaza Hotel|  
CY- San Diego, California|  
CN- United States/USA/Etats Unis/US/North America/Nord Amerika/Amerique du Nord|  
CC- C1906|  
ON- IBC USA Conferences, Inc. James Prudhomme, Life Sciences|  
A1- 1 Research Drive, Suite 400A|  
CT- Westborough, MA 01581-5195|  
OC- USA|  
TE- +1 (508) 616-5550|  
FX- +1 (508) 616-5522|  
EM- jprudhomme@ibcusa.com|  
UR- <ORGANIZER>http://www.ibcusa.com|  
DE- pharmaceutical industry, pharmaceutical research, biotechnology, protein arrays, peptide arrays, rapid screening, rapid detection applications, high throughput screening. HTS|

PC- P8030 -- Pharmacy and Pharmacology/Pharmazie und Pharmakologie/  
Pharmacie et Pharmacologie; P85121 -- Biochemistry and Biotechnology/  
Biochemie und Biotechnologie/Biochimie et Biotechnologie|  
PD- February 11, 2001|

3/4/76 (Item 1 from file: 501)  
DIALOG(R)File 501:Extel Intl News Cards  
(c) 2001 Extel Financial Inc. All rts. reserv.

00658866

Publication Date: September 12, 2000

Oxford GlycoSciences PLC  
Country: UNITED KINGDOM (GBR)

#### ACTIVITIES

Oxford GlycoSciences PLC  
Activities

Company and Cambridge Antibody Technology ("CAT") announced collaboration to develop "protein chip" technology. Technology is for detection of proteins using antibody based microarrays

Companies will combine their respective technologies, to develop new protein detection and screening technology based on antibody microarrays. Company's human protein libraries and CAT's human antibody libraries will be analysed, paired proteins and antibodies selected and microarrays developed based on Company's current protein microarray prototype format. Goal is to create new generation of protein detection technology with speed, throughput and sensitivity to serve development of research tools, diagnostics and novel therapeutics. Each party will fund its own research contribution.

Section Heading(s): Activities

3/4/77 (Item 2 from file: 501)  
DIALOG(R)File 501:Extel Intl News Cards  
(c) 2001 Extel Financial Inc. All rts. reserv.

00658865

Publication Date: September 19, 2000

Oxford GlycoSciences PLC  
Country: UNITED KINGDOM (GBR)

#### ACTIVITIES

Oxford GlycoSciences PLC  
Activities

Company and Packard BioScience Company announced collaboration to apply respective technologies to develop protein biochips ("microarrays").

Section Heading(s): Activities

3/4/78 (Item 3 from file: 501)  
DIALOG(R)File 501:Extel Intl News Cards  
(c) 2001 Extel Financial Inc. All rts. reserv.

00638481

Publication Date: September 12, 2000

Cambridge Antibody Technology Group PLC  
Country: UNITED KINGDOM (GBR)

#### ACTIVITIES

Cambridge Antibody Technology Group PLC  
Activities

Company announced strategic research collaboration with Oxford

GlycoSciences PLC.

Research collaboration is to develop "Protein Chip" technology for detection of **proteins** using antibody based **microarrays**. Company and OGS will combine their respective world-leading technologies, to develop new **protein** detection and screening technology based on antibody **microarrays**. OGS's human **protein** libraries and Company's human antibody libraries will be analysed, paired **proteins** and antibodies selected and **microarrays** developed based on OGS's current **protein microarray** prototype format. Goal is to create new generation of **protein** detection technology, with speed, throughput and sensitivity to serve development of research tools, diagnostics and novel therapeutics. Each party will fund its own research contribution.

Section Heading(s): Activities

3/4/79 (Item 1 from file: 519)  
DIALOG(R) File 519:D&B-Duns Finan.Records Plus(TM)  
(c) 2001 Dun & Bradstreet. All rts. reserv.

1997308

For Summary Financials, choose Format 14 - Price = \$33.40

Enter REPORT Sn/BIR, REPORT Sn/SER or REPORT Sn/PAR to receive special reports directly from D&B (see HELP BIR, HELP RATES 516)

3/4/80 (Item 1 from file: 660)  
FN- DIALOG(R) File 660:Federal News Service|  
CZ- (c) 2001 Federal News Service. All rts. reserv.|  
SF- Daybook|  
TI- GENERAL NEWS EVENTS - PART 2

MONDAY, NOVEMBER 15, 1999|  
SH- Final Daily Schedule|  
DL- Washington dateline general news|  
DA- 991115|  
WD- 000806|  
LI- 00073|  
LB- FEDERAL NEWS SERVICE|  
TX- dest+ dbgen,dbcon,dbscitech,dbdefense

EVENT: WORKSHOPS - NATIONAL ACADEMY OF SCIENCES (NAS) COMMISSION  
ON ENGINEERING AND TECHNICAL SYSTEMS DIVISION ON MILITARY  
SCIENCE AND TECHNOLOGY

SUBJECT: National Academy of Sciences (NAS) Commission on  
Engineering and technical Systems Division on Military  
Science and Technology holds a workshop on "Alternative  
Technologies to Replace Anti-Personnel Landmines."  
(November 15-16)

Highlights:

-- 9 am - Closed Session  
-- 1:30 pm - George Bugliarello, chairman, Committee on  
Alternative Technologies to Replace Anti-Personnel  
Landmines, Welcoming Remarks  
-- 1:45 pm - "Staffing for Study"  
-- 1:50 pm - "Administrative Procedures"  
-- 2 pm - "background for Study"  
-- 3 pm - "Review Statement of Task"  
-- 3:30 pm - "Strategic and Tactical Landmine Usage  
Overview"  
-- 5 pm - Committee Organization/Chairman's Time

LOCATION: Sheraton National Hotel, Columbia Pike and Washington  
Boulevard, Arlington, VA  
-- November 15, 1999

CONTACT: Christina Maiers, 202-334-2644 or <http://www.nas.edu>

-----  
dest+ dbgen,dbcon,dbhlth,dbmedi

EVENT: CONFERENCE - CAMBRIDGE HEALTHTECH INSTITUTE (CHI)

SUBJECT: Cambridge Healthtech Institute (CHI) holds a conference on  
"Protein Structure." (November 15-16)

Highlights:

- 8:30 am - Dr. Min S. Parker, technical staff member,  
Los Alamos National Laboratory, Opening Remarks
- 8:40 am - Dr. Wayne A. Hendrickson, biochemistry  
professor, Howard Hughes Medical Institute (HHMI) Research  
Laboratories, and biochemistry and molecular biophysics  
department, Columbia University, Keynote Address:  
"Structure Methods Becoming Structural Genomics"
- 9:15 am - "Structural Proteomics: A method of Target  
Validation and Invalidation"
- 10:15 am - "Structural genomics and Automated Structure  
Selection"
- 10:45 am - "The Role of NMR in Structural Genomics"
- 11:15 am - Panel Discussions
- 1:30 pm - Dr. John Moulton, computational biology  
professor, Center for Advanced Research in Biotechnology,  
University of Maryland Biotechnology Institute
- 1:35 pm - "Computational Approaches to Structural  
Genomics"
- 2:05 pm - "Large-Scale Comparative Protein Modeling"
- 2:35 pm - "Comparative Protein Structure Modeling in  
Genomics"
- 3:45 pm - "Proteome Databases for Applications of Model  
Organism Knowledge to Nonmodel Organisms"
- 4:15 pm - "Structure-Based Assignment of Molecular  
Functions of Hypothetical Proteins"
- 4:45 pm - "Deriving Function From Protein Structure  
Modeling"
- 5:15 pm - Panel Discussion

LOCATION: Capital Hilton Hotel, 16th and K Streets NW, Washington,  
DC

-- November 15, 1999

CONTACT: Jennifer Laakso, 617-630-1385 or  
<http://www.healthtech.com/conference/pst/pst.htm>

-----  
dest+ dbgen,dbcon,dbhlth,dbmedi,dbscitech

EVENT: CONFERENCE - CAMBRIDGE HEALTHTECH INSTITUTE (CHI)

SUBJECT: Cambridge Healthtech Institute (CHI) holds a conference on  
"Chemokine and Chemokine Receptors: Disease Targets for  
Therapeutic Development." (November 15-16)

Highlights:

- 9 am - Dr. Thomas J. Schall, president and CEO,  
Chemocentryx Inc., Opening Comments
- 9:10 am - "Chemokines at the dawn of the 21st Century:  
Aliens and Archetypes"
- 9:50 am - "The ELR-CXC-Chemokine Platelet Basic Protein  
(PBP) Potently Desensitizes Chemokine-Induced Neutrophil  
Activation"
- 10:45 am - "Changes in Chemokine Receptor Expression  
Patterns During T Cell Development"
- 11:15 am - "CCR4 Knockout Mouse Models of Lung  
Inflammation"
- 11:45 pm - Panel Discussion
- 1:40 pm - Dr. Joseph Hesselgesser, research scientist,  
Immunology, Berlex BioSciences

- 1:45 pm - "Mechanisms of Leukocyte Trafficking Across the Blood Brain Barrier"
- 2:15 pm - "Chemokines and Their Receptors in Multiple Sclerosis"
- 2:45 pm - "Chemokine and Chemokine Receptor Expression Patterns Regulate the Pathogenesis of Autoimmune Encephalomyelitis"
- 3:45 pm - "Inhibition of Angiogenesis Induced by IP-10/CRG-2"
- 4:15 pm - "Chemokines and Their Receptors in Allograft Rejection"
- 4:45 pm - Panel Discussion

LOCATION: Ritz Carlton Tysons Corner Hotel, 1700 Tysons Boulevard, McLean, VA  
 -- November 15, 1999

CONTACT: Jennifer Laakso, 617-630-1385 or  
<http://www.healthtech.com/conference/kmo/kmo.htm>

-----  
 dest+ dbgen,dbcon,dbdepten,dbscitech  
 EVENT: CONFERENCE - ENERGY DEPARTMENT OAK RIDGE NATIONAL  
 LABORATORY (ORNL) ENVIRONMENTAL SCIENCES DIVISION

SUBJECT: Energy Department Oak Ridge National Laboratory (ORNL)  
 Environmental Sciences Division holds its "Seventh Annual  
 Conference on Small Genomes." (November 14-17)

Highlights:

- 8 am - "Functional Analysis of the Vibrio Cholerae Genome"
- 8:45 am - "Printing Technologies for Genomic Microarrays"
- 9:15 am - "Advances in Microarray Scanning and Analysis"
- 10 am - "Flowthrough Geosensor Chips and Their Application in Microbial Genomics"
- 10:30 am - High Throughput, Low Cost Oligonucleotides for Gene Expression Analysis"
- 11:30 am - "Whole-Genome RNA and Protein Regulatory Networks in E. Coli and S. Cerevisiae"
- 1 pm - "Gene Transfer Systems for Extremophiles"
- 1:30 pm - "Essential and Dispensable Genes in Mycoplasmas"
- 2:30 pm - "Rapid Identification and Precise Expression Profiling of Proteomes by Mass Spectrometry"
- 3:15 pm - "Exploration of Genome Usage in Methanococcus Jannaschii and Pyrococcus Furiosus From the Protein Point-of-View"
- 4:15 pm - "Using Genomics Databases to Understand Protein Expression Profiles of M. Tuberculosis"
- 5 pm - Poster Session

LOCATION: Doubletree Hotel, 300 Army Navy Drive, Arlington, VA  
 -- November 15, 1999

CONTACT: Kim Smith, 423-576-4860 or  
<http://www.esd.ornl.gov/programs/microbes/agenda2.html>

ID- daybook; gen; II; mon|  
 DE- General News Events, Conventions, conferences, symposiums, forums,  
 Science and technology, Defense; General News Events, Conventions,  
 conferences, symposiums, forums, Health, Medicine (technology); General  
 News Events, Conventions, conferences, symposiums, forums, Health,  
 Medicine (technology), Science and technology; General News Events,  
 Conventions, conferences, symposiums, forums, Dept. of Energy, DOE,  
 Science and technology|  
 ?logoff hold

3/4/14 (Item 13 from file: 5)  
 FN- DIALOG(R)File 5:Biosis Previews(R)|  
 CZ- (c) 2001 BIOSIS. All rts. reserv.|  
 AZ- 12522220|  
 AA- 200000275722|  
 TI- Chemical microarray system to phenotype the protein kinase  
 enzymatic profile of cell lines and tissues.|  
 AU- Lam Kit S(a); Falsey James; Manat Renil; Park Steven|  
 CS- (a)Arizona Cancer Ctr, Tucson, AZ USA|  
 JN- Proceedings of the American Association for Cancer Research Annual  
 Meeting|  
 IS- 41|  
 PG- 850|  
 DA- March,|  
 PY- 2000|  
 ME- print.|  
 CT- 91st Annual Meeting of the American Association for Cancer Research.|  
 LO- San Francisco, California, USA|  
 DA- April 01-05, 2000|  
 SN- 0197-016X|  
 RT- Citation|  
 LA- English|  
 SL- English|  
 RN- 9026-43-1: PROTEIN KINASE|  
 DE- <MAJOR CONCEPT> Tumor Biology|  
 DE- <BIOSYSTEMATIC> Animalia|  
 DE- <ORGANISMS> animal (Animalia)--animal model|  
 DE- <SUPER TAXA> Animals|  
 DE- <DISEASES> cancer--in-vitro cell study, neoplastic disease, tissue  
 sample study|  
 DE- <CHEMICALS> protein kinase--enzymatic profile phenotype, tumor cell  
 activity, tumor tissue activity|  
 DE- <METHODS> chemical microarray system--analytical method, genetic method  
 |  
 DE- <MISC.> Meeting Abstract|  
 DE- <ALT. INDEX> Neoplasms (MeSH)|  
 CC- 10802 Enzymes-General and Comparative Studies; Coenzymes  
 24004 Neoplasms and Neoplastic Agents-Pathology; Clinical Aspects;  
 Systemic Effects  
 00520 General Biology-Symposia, Transactions and Proceedings of  
 Conferences, Congresses, Review Annuals|  
 BC- 33000 Animalia-Unspecified|

3/4/22 (Item 21 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 12122478|  
AA- 199900417327|  
TI- Novel chemistry for parallel synthesis of microarrays of  
oligonucleotides and peptides using photogenerated acids.|  
AU- Gao Xiaolian(a); LeProust Eric M(a); Pellois Jean Philippe(a); Yu  
Peilin(a); Zhang Hua(a); Wang Wei; Zhou Xiaochuan|  
CS- (a)Department of Chemistry, University of Houston, 4800 Calhoun Rd,  
Houston, TX, 77204-5641 USA|  
JN- Abstracts of Papers American Chemical Society|  
VO- 218|  
IS- 1-2|  
PG- MEDI 3|  
PY- 1999|  
CT- 218th National Meeting of the American Chemical Society, Parts 1 and 2|  
LO- New Orleans, Louisiana, USA|  
DA- August 22-26, 1999|  
SP- American Chemical Society; |  
SN- 0065-7727|  
RT- Citation|  
LA- English|  
DE- <MAJOR CONCEPT> Chemistry; Methods and Techniques|  
DE- <CHEMICALS> oligonucleotides--microarrays, synthesis; photogenerated  
acids--reagent|  
DE- <METHODS> chemical synthesis--Synthesis/Modification Techniques,  
synthetic method|  
DE- <MISC.> Meeting Abstract|  
CC- 10060 Biochemical Studies-General  
10050 Biochemical Methods-General  
00520 General Biology-Symposia, Transactions and Proceedings of  
Conferences, Congresses, Review Annuals|

3/4/23 (Item 22 from file: 5)

FN- DIALOG(R)File 5:Biosis Previews(R)|  
CZ- (c) 2001 BIOSIS. All rts. reserv.|  
AZ- 11994187|  
AA- 199900274706|  
TI- Protein microarrays for gene expression and antibody screening.|  
AU- Lueking Angelika(a); Horn Martin(a); Eickhoff Holger(a); Buessow Konrad  
(a); Lehrach Hans(a); Walter Gerald(a)|  
CS- (a)Max Planck Institute for Molecular Genetics, Ihnestrass 73,  
D-14195, Berlin Germany|  
JN- Analytical Biochemistry|  
VO- 270|  
IS- 1|  
PG- 103-111|  
DA- May 15, |  
PY- 1999|  
SN- 0003-2697|  
DT- Article|  
RT- Abstract|  
LA- English|  
SL- English|  
AB- Proteins translate genomic sequence information into function, enabling  
biological processes. As a complementary approach to gene expression  
profiling on cDNA microarrays, we have developed a technique for  
high-throughput gene expression and antibody screening on chip-size  
protein microarrays. Using a picking/spotting robot equipped with a new  
transfer stamp, protein solutions were gridded onto polyvinylidene  
difluoride filters at high density. Specific purified protein was  
detected on the filters with high sensitivity (250 amol or 10 pg of a  
test protein). On a microarray made from bacterial lysates of 92 human  
cDNA clones expressed in a microtiter plate, putative protein  
expressors could be reliably identified. The rate of false-positive  
clones, expressing proteins in incorrect reading frames, was low.  
Product specificity of selected clones was confirmed on identical



microarrays using monoclonal antibodies. Cross-reactivities of some antibodies with unrelated proteins imply the use of protein microarrays for antibody specificity screening against whole libraries of proteins. Because this application would not be restricted to antigen-antibody systems, protein microarrays should provide a general resource for high-throughput screens of gene expression and receptor-ligand interactions.

- DE- <MAJOR CONCEPT> Methods and Techniques; Molecular Genetics  
(Biochemistry and Molecular Biophysics)|
- DE- <BIOSYSTEMATIC> Hominidae--Primates, Mammalia, Vertebrata, Chordata,  
Animalia|
- DE- <ORGANISMS> human (Hominidae)|
- DE- <SUPER TAXA> Animals; Chordates; Humans; Mammals; Primates; Vertebrates  
|
- DE- <CHEMICALS> antibodies--screening; antigen-antibody systems; cDNA {  
complementary DNA}--cloning; ligands; protein microarrays--analysis,  
applications; proteins--analysis|
- DE- <METHODS> antibody detection--Detection/Labeling Techniques, analytical  
method; antibody screening--Analysis/Characterization Techniques--CB,  
analytical method; image analysis--imaging method, imaging techniques;  
protein purification--Isolation/Purification Techniques--CB,  
purification method|
- DE- <MISC.> biological processes; gene expression--analysis;  
receptor-ligand interactions--analysis; robotics|
- CC- 10054 Biochemical Methods-Proteins, Peptides and Amino Acids
- 03502 Genetics and Cytogenetics-General
- 10050 Biochemical Methods-General
- 10064 Biochemical Studies-Proteins, Peptides and Amino Acids
- 10506 Biophysics-Molecular Properties and Macromolecules
- 34502 Immunology and Immunochemistry-General; Methods
- 13002 Metabolism-General Metabolism; Metabolic Pathways
- 10300 Replication, Transcription, Translation
- 10060 Biochemical Studies-General
- 10052 Biochemical Methods-Nucleic Acids, Purines and Pyrimidines|
- BC- 86215 Hominidae|

08276786 Supplier Number: 69846280 (THIS IS THE FULLTEXT)  
IBC Presents the First Conference Dedicated to Protein Microarray  
Technology.

PR Newswire, p4379

Feb 2, 2001

TEXT:

WESTBOROUGH, Mass., Feb. 2 /PRNewswire/ --

IBC USA organizes Protein Microarray Technology: From Proteomics  
Discovery to Diagnostics -- Expectations and Limitations. The event will  
be held on March 21-23, 2001 in San Diego, CA.

Microarray technology allows the simultaneous analysis of thousands  
of molecular parameters with a single experiment. Today, methods and  
techniques of the DNA chip field are being effectively transferred to  
protein array technology. This event allows attendees to learn about where  
this new, exciting field is at right now, where it is moving, and how  
proteomics, drug discovery, and diagnostics are being revolutionized by  
protein microtechnology. This event aims to accelerate discussion of The  
Proteome Project and examine the technologies being developed and how they  
will impact research.

Conference highlights include:

\*Keynote address by Dr. Roger Ekins - a founding father of  
microtechnology

- \*Data from Beta Tests, as well as Commercial Applications
- \*How to make and use protein/peptide arrays
- \*Direct application of technology in diagnostics
- \*Proteomics discovery research and assay development
- \*Latest technologies in the protein array field
- \*First-time presentations of case study data/results
- \*Bridging the gap between genomics and proteomics

For more details on this, visit our website:

<http://www.ibcusa.com/2623>.

CONTACT:

Abby Votto

IBC USA Conferences

One Research Drive

P.O. Box 5195

Suite 400A

Westborough, MA 01581-5195

[avotto@ibcusa.com](mailto:avotto@ibcusa.com)

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\*File 73: For information about Explode feature please  
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(c)2001 Japan Science and Tech Corp(JST)

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the current months data. See Help News94 for details.

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File 99:Wilson Appl. Sci & Tech Abs 1983-2001/Mar

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File 100:Market Guide Company Financials 2001/Apr 30  
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 File 107:Adis R&D Insight 1986-2001/Apr W3  
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 File 111:TGG Natl.Newspaper Index(SM) 1979-2001/Apr 25  
     (c) 2001 The Gale Group  
 File 115:Research Centers & Services 1994-2000/Nov  
     (c) 2000 Gale Research Inc.  
 File 133:S&P's Corp.Descrip.+News 2001/Apr 30  
     (c) 2001 McGraw-Hill Co. Inc  
 File 143:Biol. & Agric. Index 1983-2001/Mar  
     (c) 2001 The HW Wilson Co  
 File 144:Pascal 1973-2001/Apr W5  
     (c) 2001 INIST/CNRS  
 File 148:Gale Group Trade & Industry DB 1976-2001/Apr 27  
     (c)2001 The Gale Group  
 File 149:TGG Health&Wellness DB(SM) 1976-2001/Apr W4  
     (c) 2001 The Gale Group  
 File 151:HealthSTAR 1975-2000/Dec  
     (c) format only 2000 The Dialog Corporation  
 \*File 151: Final updates for this file have been loaded and the  
 file is now closed. Please see Help News151 for changes to the file.  
 File 155:MEDLINE(R) 1966-2001/May W3  
     (c) format only 2000 Dialog Corporation  
 \*File 155: Medline has now updated. For further information  
 see Help News155.  
 File 159:Cancerlit 1975-2001/Mar  
     (c) format only 2001 Dialog Corporation  
 File 165:EventLine(TM) 1990-2001/Mar  
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 \*File 165: Updates are currently restricted  
 to Medical and Biotechnical events only.  
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 File 174:Pharm-line(R) 1978-2001/Apr W4  
     (c) CROWN COPYRIGHT 2001  
 \*File 174: UDs have been readjusted to reflect the current months data.  
 There is no data missing.  
 File 286:Biocommerce Abs.& Dir. 1981-2001/Apr B1  
     (c) 2001 BioCommerce Data Ltd.  
 File 342:Derwent Patents Citation Indx 1978-01/200120  
     (c) 2001 Derwent Info Ltd  
 \*File 342: Price changes as of 1/1/01. Please see HELP RATES 342.  
 File 351:Derwent WPI 1963-2001/UD,UM &UP=200123  
     (c) 2001 Derwent Info Ltd  
 \*File 351: Price changes as of 1/1/01. Please see HELP RATES 351.  
 72 Updates in 2001. Please see HELP NEWS 351 for details.  
 File 357:Derwent Biotechnology Abs 1982-2001/May B1  
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 \*File 420: Please check rates (enter r from the main menu) for  
 important information about patent collections and availability.  
 File 431:MediConf: Medical Con. & Events 1998-2001/Apr B2  
     (c) 2001 Dr. R. Steck  
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 File 440:Current Contents Search(R) 1990-2001/May W1  
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 File 453:Drugs of the Future 1990-2001/Mar  
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 File 484:Periodical Abstracts Plustext 1986-2001/Apr W4

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 (c) 2001 Dun & Bradstreet  
 \*File 519: Enter REPORT Sn/BIR, or REPORT Sn/SER, or REPORT Sn/PAR  
 to receive special reports directly from D&B.  
 File 613:PR Newswire 1999-2001/Apr 30  
 (c) 2001 PR Newswire Association Inc  
 \*File 613: File 613 now contains data from 5/99 forward.  
 Archive data (1987-4/99) is available in File 813.  
 File 621:Gale Group New Prod.Annou.(R) 1985-2001/Apr 27  
 (c) 2001 The Gale Group  
 File 649:Gale Group Newswire ASAP(TM) 2001/Apr 25  
 (c) 2001 The Gale Group  
 File 660:Federal News Service 1991-2001/Mar 08  
 (c) 2001 Federal News Service  
 \*File 660: This file is temporarily not updating.

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3/5/35 (Item 6 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

(c) 2001 Inst for Sci Info. All rts. reserv.

08990817 Genuine Article#: 353PF Number of References: 3

Title: Protein microarrays hit the spot

Author(s): Borman S

Journal: CHEMICAL & ENGINEERING NEWS, 2000, V78, N37 (SEP 11), P6-7

ISSN: 0009-2347 Publication date: 20000911

Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036

Language: English Document Type: NEWS ITEM

Journal Subject Category: ENGINEERING, CHEMICAL

Cited References:

MACBEATH G, 1999, V121, P7967, J AM CHEM SOC

MACBEATH G, 2000, V122, P7849, J AM CHEM SOC

SCHREIBER SL, 2000, V289, P1760, SCIENCE

?t 3/5/38

3/5/38 (Item 9 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

(c) 2001 Inst for Sci Info. All rts. reserv.

07495706 Genuine Article#: 173NX Number of References: 34

Title: Create a protein microarray using a hydrogel ''stamper''

Author(s): Gaber BP (REPRINT); Martin BD; Turner DC

Corporate Source: USN,RES LAB, LAB MOL INTERFACIAL

INTERACT/WASHINGTON//DC/20375 (REPRINT); GEORGE MASON UNIV,CTR COMP SCI

& INFORMAT/FAIRFAX//VA/22030; USN,RES LAB, CTR BIOMOL SCI &

ENGN/WASHINGTON//DC/20375

Journal: CHEMTECH, 1999, V29, N3 (MAR), P20-24

ISSN: 0009-2703 Publication date: 19990300

Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036

Language: English Document Type: ARTICLE

Geographic Location: USA

Subfile: CC PHYS--Current Contents, Physical, Chemical & Earth Sciences; CC

ENGI--Current Contents, Engineering, Computing & Technology;

Journal Subject Category: CHEMISTRY, APPLIED

Abstract: The keys to fabricating multiplexed biosensors are the capability

to lay down biomolecules with spatial fidelity retention of activity,

and an absolute minimum of biochemical cross-talk. The hydrogel stamper does this by delivering a molecular film of protein directly onto the surface in a single step.

Identifiers--Keyword Plus(R): SURFACES; DNA; FABRICATION; BIOSENSORS; ANTIBODIES; CELLS; GOLD

Cited References:

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BAKER DA, 1988, V29, P691, POLYMER  
BHATIA SK, 1992, V114, P4432, J AM CHEM SOC  
BRITLAND S, 1992, V8, P155, BIOTECHNOL PROGR  
BURNS MA, 1998, V282, P484, SCIENCE  
BYFIELD MP, 1994, V9, P373, BIOSENS BIOELECTRON  
CHEE M, 1996, V227, P610, SCIENCE  
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GUSCHIN D, 1997, V250, P203, ANAL BIOCHEM  
JANATA J, 1989, PCH1, PRINCIPLES CHEM SENS  
KAZANSKI KS, 1993, V104, P97, ADV POLYM SCI  
KIMURA J, 1988, V4, P41, BIOSENSORS  
KUMAR A, 1993, V63, P2002, APPL PHYS LETT  
LI RH, 1996, V50, P365, BIOTECHNOL BIOENG  
LOPEZ GP, 1993, V115, P5877, J AM CHEM SOC  
LU B, 1996, V121, PR29, ANALYST  
LUNSTROM I, 1987, P201, POLYM SURFACES INTER  
MARIUZZA RA, 1987, V16, P139, ANNU REV BIOPHYS BIO  
MARKOWITZ MA, 1997, V68, P57, APPL BIOCHEM BIOTECH  
MARTIN BD, 1998, V19, P69, BIOMATERIALS  
MARTIN BD, 1998, V14, P3971, LANGMUIR  
MARTIN BD, 1992, V25, P7081, MACROMOLECULES  
PADDLE BM, 1996, V11, P1079, BIOSENS BIOELECTRON  
PEPPAS NA, 1986, HYDROGELS MED PHARM  
PRITCHARD DJ, 1995, V67, P3605, ANAL CHEM  
ROGERS KR, 1996, V30, P486, ENVIRON SCI TECHNOL  
SEFTON MV, 1993, V107, P143, ADV POLYM SCI  
SINGHVI R, 1994, V264, P696, SCIENCE  
WEN AC, 1996, V50, P357, BIOTECHNOL BIOENG

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DIALOG(R)File 73:EMBASE

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11011719 EMBASE No: 2001051263

**Advances in protein microarray technology for protein expression and interaction profiling**

Haab B.B.

B.B. Haab, Van Andel Institute, 333 Bostwick NE, Grand Rapids, MI 49503  
United States

AUTHOR EMAIL: brian.haab@vai.org

Current Opinion in Drug Discovery and Development ( CURR. OPIN. DRUG  
DISCOV. DEV. ) (United Kingdom) 2001, 4/1 (116-123)

CODEN: CODDF ISSN: 1367-6733

DOCUMENT TYPE: Journal ; Review

LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

NUMBER OF REFERENCES: 39

Protein microarrays address a great demand for high-throughput protein analysis techniques. Because protein microarrays detect many proteins in parallel, are quantitative, and have minimal reagent and sample consumption

requirements due to miniaturization, they are potentially powerful tools for applications in basic and applied biology. Advances in manufacturing, protein immobilization and detection methods have enabled high-throughput protein quantitation and interaction studies. Protein microarrays can be applied to protein function studies, screening the production of antibodies and recombinant proteins, discovery of proteins implicated in disease or that are potential drug targets, and rapid detection or diagnosis of disease. A remaining challenge for the full implementation of protein microarrays is the acquisition of large sets of high-affinity and highly specific protein capture reagents.

DRUG DESCRIPTORS:

\*protein

MEDICAL DESCRIPTORS:

\*fluorescence; \*protein analysis; \*electronics

protein expression; protein immobilization; drug manufacture; screening;

DNA microarray; review

CAS REGISTRY NO.: 67254-75-5 (protein)

SECTION HEADINGS:

029 Clinical and Experimental Biochemistry

3/5/43 (Item 1 from file: 98)

DIALOG(R)File 98:General Sci Abs/Full-Text

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04389682 H.W. WILSON RECORD NUMBER: BGSA00139682

Protein microarrays.

Analytical Chemistry (Anal Chem) v. 72 no21 (Nov. 1 2000) p. 677A

SPECIAL FEATURES: il ISSN: 0003-2700

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

RECORD TYPE: Abstract RECORD STATUS: Corrected or revised record

ABSTRACT: Researchers at Harvard University, Massachusetts, have applied new DNA microarray technology to proteins. MacBeath and Schreiber used their adapted system, reported in a recent Science (2000;289:1760-3), to assay protein-protein interactions, to screen for enzyme substrates, and to study protein-small molecule experiments.

DESCRIPTORS:

Proteins--Analysis; DNA chips

3/5/52 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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10591540 20473216

Picture story. A protein microarray.

Feng HP

Nature structural biology (UNITED STATES) Oct 2000, 7 (10) p829,

ISSN 1072-8368 Journal Code: B98

Languages: ENGLISH

Document type: Journal Article

Subfile: INDEX MEDICUS

Tags: Human

Descriptors: \*Proteins--chemistry--CH; Genome, Human

CAS Registry No.: 0 (Proteins)

3/5/53 (Item 1 from file: 165)

DIALOG(R)File 165:EventLine(TM)

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00495021

EVENT TITLE: The World's First Meeting on Protein Microarray  
Technology

TYPE OF EVENT: Conference

EVENT DATE(S): March 21-23, 2001  
 HOST SITE: Hilton SD Resort  
 EVENT CITY: San Diego  
 EVENT STATE: California, USA  
 EVENT COUNTRY: USA  
 REGION: North America  
 EXHIBITION: No  
 ORGANIZER: IBC USA Conferences Inc.  
 Suite 400A  
 One Research Drive, POB 5195  
 Westborough, MA 01581-5195 USA  
 TELEPHONE: (508) 616-5550  
 FAX: (508) 616-5522  
 E-MAIL: reg@ibcusa.com  
 URL: ibcusa.com/2541  
 TRANSLATION: No  
 DESCRIPTORS: Pharmaceuticals/Pharmacology 15000; Pharmaceuticals - General  
 15010; Biotechnology 18000; Biotechnology - General 18010;  
 Biology 19000; Genetics 19030; Immunology 19040; Microbiology  
 19050; Chemicals 46000; Drugs and Pharmaceuticals 46040  
 RECORD INPUT DATE: 010212

3/5/58 (Item 4 from file: 286)  
 DIALOG(R)File 286:Biocommerce Abs.& Dir.  
 (c) 2001 BioCommerce Data Ltd. All rts. reserv.

00189693 Journal Announcement: 20000927 Doc Type: 1 Status: Revised  
 Oxford GlycoSciences Press Release, 12SEP2000  
 CAT Press Release, 12SEP2000  
 BioWorld International, 20SEP2000, Vol(No) 5(39), Page(s) 1,2  
 Oxford GlycoSciences (OGS) and Cambridge Antibody Technology (CAT) are to  
 co-develop a **protein chip** technology for the detection of **proteins**  
 using antibody based **microarrays**. Each company will fund its own  
 research.  
 COMPANY/ORGANIZATION NAME(S):  
 Oxford GlycoSciences plc (OGS), UK (37757)  
 Cambridge Antibody Technology Group plc (CAT), UK (41897)

3/5/64 (Item 2 from file: 351)  
 DIALOG(R)File 351:Derwent WPI  
 (c) 2001 Derwent Info Ltd. All rts. reserv.

013540964 \*\*Image available\*\*  
 WPI Acc No: 2001-025170/200103  
 XRAM Acc No: C01-007783  
 Producing microarray chip of chemical compounds e.g. DNAs, peptide  
 nucleic acids and polypeptides for use in biological assay, drug  
 screening and gene sequence analysis, with high space resolution and  
 accuracy, at low cost  
 Patent Assignee: LU Z (LUZZ-I)  
 Inventor: HE N; LU Z; ZHAO Y; MA J  
 Number of Countries: 091 Number of Patents: 003  
 Patent Family:  

Patent No	Kind	Date	Applicat No	Kind	Date	Week
WO 200071746	A1	20001130	WO 2000CN125	A	20000519	200103 B
AU 200045354	A	20001212	AU 200045354	A	20000519	200115
CN 1274758	A	20001129	CN 99106790	A	19990521	200121

Priority Applications (No Type Date): CN 99106790 A 19990521  
 Patent Details:

Patent No Kind Lan Pg Main IPC Filing Notes  
 WO 200071746 A1 C 23 C12Q-001/68

Designated States (National): AE AL AM AT AU AZ BA BB BG BR BY CA CH CR  
 CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR  
 KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG  
 SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW



Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR  
IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW  
AU 200045354 A C12Q-001/68 Based on patent WO 200071746  
CN 1274758 A C12Q-001/68

Abstract (Basic): WO 200071746 A1

NOVELTY - A method for producing a microarray chip of chemical compounds, is new.

DETAILED DESCRIPTION - A method for producing a microarray chip of chemical compounds comprises:

(a) designing a microarray chip of the chemicals according to requirements and preparing the specific micro reaction chamber;

(b) immobilizing the reaction chamber onto a substrate with a fixed point device to form a micro reaction chamber fixed point synthesis system;

(c) introducing a reaction solution to the synthesis system for chemical reaction on the substrate surface after contacting with the micro reaction chamber; and

(d) controlling the fixed point synthesis by changing the reaction chamber, or by regulating the flow of liquid in the reaction chamber to provide a microarray chip formed with various compounds on the substrate.

USE - The method is for producing microarray chip of chemical compounds, particularly DNA, peptide nucleic acid and polypeptide microarray chips (claimed) for use in biological assay, diagnosis, drug screening, gene sequence analysis and combinatorial synthesis.

ADVANTAGE - The chips are reliable, have high space resolution and accuracy, and are produced at a low cost.

DESCRIPTION OF DRAWING(S) - Structure of a micro reaction chamber.  
pp; 23 DwgNo 1/13

Title Terms: PRODUCE; CHIP; CHEMICAL; COMPOUND; PEPTIDE; NUCLEIC; ACID;  
BIOLOGICAL; ASSAY; DRUG; SCREEN; GENE; SEQUENCE; ANALYSE; HIGH; SPACE;  
RESOLUTION; ACCURACY; LOW; COST

Derwent Class: B04; D16

International Patent Class (Main): C12Q-001/68

International Patent Class (Additional): G01N-033/15

File Segment: CPI

3/5/65 (Item 3 from file: 351)

DIALOG(R)File 351:Derwent WPI

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013500822 \*\*Image available\*\*

WPI Acc No: 2000-672763/200065

XRAM Acc No: C00-203826

XRPX Acc No: N00-498763

Simultaneous detection of many protein-binding ligands, useful e.g. for diagnosis and drug screening, uses high-density microarray of immobilized peptides

Patent Assignee: UNIV LELAND STANFORD JUNIOR (STRD )

Inventor: BROWN P; HAAB B

Number of Countries: 021 Number of Patents: 001

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
WO 200063701	A2	20001026	WO 2000US10171	A	20000414	200065 B

Priority Applications (No Type Date): US 99129449 A 19990415

Patent Details:

Patent No Kind Lan Pg Main IPC Filing Notes

WO 200063701 A2 E 33 G01N-033/68

Designated States (National): CA JP US

Designated States (Regional): AT BE CH CY DE DK ES FI FR GB GR IE IT LU  
MC NL PT SE

Abstract (Basic): WO 200063701 A2

NOVELTY - Simultaneous detection of many protein-binding ligands  
(I) in a sample comprises contacting the sample with a microarray of peptides, washing to remove unbound sample and detecting bound

(I). The microarray has at least 100 discrete regions, carrying different (I), per cm<sup>2</sup> of solid planar support.

USE - The method is particularly used to detect and quantify antigens or antibodies, but may also be used to detect any protein-interacting compound, e.g. a polynucleotide, hormone, vitamin, co-factor etc., e.g. in large scale diagnostic assays (for detecting disease markers, or for assessing response to drugs or environmental factors); for drug development and generally in molecular biology, immunology and toxicology.

ADVANTAGE - The miniaturized microarray uses far less sample than traditional immunoassays and analysis of many (I) in parallel allows diagnosis to be made from a multidimensional pattern of expression. Where measurements are taken by comparative fluorescence, greater precision over a wide range of ligand concentrations and binding affinities is achieved, compared with measuring the absolute amount of bound ligand.

DESCRIPTION OF DRAWING(S) - The figure shows a side view of a reagent-dispensing device having an open-capillary dispensing head.  
pp; 33 DwgNo 1/8

Title Terms: SIMULTANEOUS; DETECT; PROTEIN; BIND; LIGAND; USEFUL; DIAGNOSE;  
DRUG; SCREEN; HIGH; DENSITY  
Derwent Class: B04; D16; S03  
International Patent Class (Main): G01N-033/68  
File Segment: CPI; EPI

3/5/66 (Item 4 from file: 351)

DIALOG(R) File 351:Derwent WPI

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013456333

WPI Acc No: 2000-628276/200060

XRAM Acc No: C00-188263

Identifying clones encoding membranal or secreted proteins for identifying targets of drug development, by deriving probes from membrane-bound and free polysomes and performing microarray-based comparison

Patent Assignee: QUARK BIOTECH INC (QUAR-N); KOHN K I (KOHN-I)

Inventor: EINAT P; MOR O; NOVAK L; SKALITER R

Number of Countries: 090 Number of Patents: 002

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
WO 200056935	A1	20000928	WO 2000US7830	A	20000324	200060 B
AU 200039168	A	20001009	AU 200039168	A	20000324	200103

Priority Applications (No Type Date): US 99125975 A 19990324

Patent Details:

Patent No Kind Lan Pg Main IPC Filing Notes

WO 200056935 A1 E 29 C12Q-001/68

Designated States (National): AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

AU 200039168 A C12Q-001/68 Based on patent WO 200056935

Abstract (Basic): WO 200056935 A1

NOVELTY - A method for identifying clones which encode membranal or secreted proteins comprising preparing cDNA probes from total RNA extracted from membrane bound polysomes and free polysomes and microarray based comparison of the relative abundance of the different RNA species, is new.

USE - The method is useful for identifying clones encoding membranal or secreted proteins which provides an efficient tool for identifying targets of drug development. The method serves as a tool for augmenting conventional differential analysis of relative mRNA abundance in different RNA sources by virtue of its use of RNA extracted from specific subcellular compartments as templates for cDNA

probes (claimed).

ADVANTAGE - The method allows rapid identification of highly relevant membranal and secreted proteins.

pp; 29 DwgNo 0/1

Title Terms: IDENTIFY; CLONE; ENCODE; SECRETION; PROTEIN; IDENTIFY; TARGET; DRUG; DEVELOP; DERIVATIVE; PROBE; MEMBRANE; BOUND; FREE; PERFORMANCE; BASED; COMPARE

Derwent Class: B04; D16

International Patent Class (Main): C12Q-001/68

International Patent Class (Additional): C12M-003/00

File Segment: CPI

3/5/67 (Item 5 from file: 351)

DIALOG(R) File 351:Derwent WPI

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013439491

WPI Acc No: 2000-611434/200058

XRAM Acc No: C00-182920

Microarray of peptide affinity probes useful for analyzing and quantitating gene products is formed on the porous membrane proximate to an electrode

Patent Assignee: COMBIMATRIX CORP (COMB-N)

Inventor: MONTGOMERY D D; ROSSI F M

Number of Countries: 089 Number of Patents: 002

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
WO 200053625	A2	20000914	WO 2000US6676	A	20000310	200058 B
AU 200038833	A	20000928	AU 200038833	A	20000310	200067

Priority Applications (No Type Date): US 99123877 A 19990311; US 99123791 A 19990311

Patent Details:

Patent No Kind Lan Pg Main IPC Filing Notes

WO 200053625 A2 E 52 C07K-001/00

Designated States (National): AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZW

Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

AU 200038833 A C07K-001/00 Based on patent WO 200053625

Abstract (Basic): WO 200053625 A2

NOVELTY - An array (I) of peptide probes comprising a porous membrane proximate to at least one electrode, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) producing an array of peptide probes for analyzing gene products comprising:

(a) providing a substrate having at its surface at least one electrode that is proximate to an amino acid bearing a protected chemical functional group;

(b) applying a potential to the electrode sufficient to generate electrochemical reagents capable of deprotecting the protected chemical functional groups of the amino acid; and

(c) bonding the deprotected chemical functional group with another amino acid; and

(2) making an array of one or more peptide probes for analyzing gene products comprising:

(a) placing a buffering or scavenging solution in contact with an array of electrodes that is proximate to substrate surface, where the surface being proximate to one or more amino acids bearing a protected chemical functional group attached to it;

(b) selectively deprotecting a protected chemical functional group on the amino acids;

(c) bonding another amino acid having a protected chemical functional group to one or more deprotected chemical functional groups

of the amino acid; and

(d) repeating the selective deprotection of a chemical functional group on a bonded protected amino acid and subsequently bonding an additional amino acid to the deprotected chemical functional group until at least two separate peptides of desired length are formed on the substrate surface.

USE - For determining the presence or quantitating the amount of a gene product in a biological sample. (I) is also useful for identifying the functional binding domain of a gene product in a sample (claimed). The peptide probe arrays are useful for discovering new drug targets.

ADVANTAGE - The gene products that are difficult to observe by two dimensional electrophoresis such as hydrophobic, basic and large proteins can be observed. Variability resulting from differences in sample preparation and differences in susceptibility of protein to stain is reduced. Peptide probes are placed on a array defined pattern which eliminates the need for complex pattern recognition software. The peptide array allows gene product expression levels, amino acid sequence and potential interaction between the gene product and other peptide to be determined. The method significantly reduces the time required to develop new drugs.

pp; 52 DwgNo 0/12

Title Terms: PEPTIDE; AFFINITY; PROBE; USEFUL; QUANTIFICATION; GENE; PRODUCT; FORMING; POROUS; MEMBRANE; PROXIMATE; ELECTRODE

Derwent Class: B04; D16

International Patent Class (Main): C07K-001/00

File Segment: CPI

3/5/68 (Item 1 from file: 357)

DIALOG(R) File 357: Derwent Biotechnology Abs

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0261985 DBA Accession No.: 2001-00454 PATENT

Microarray of peptide affinity probes useful for analyzing and quantiating gene products is formed on the porous membrane proximate to an electrode - peptide probe

AUTHOR: Rossi F M; Montgomery D D

CORPORATE SOURCE: Burlingame, CA, USA.

PATENT ASSIGNEE: Combimatrix 2000

PATENT NUMBER: WO 200053625 PATENT DATE: 20000914 WPI ACCESSION NO.: 2000-611434 (2058)

PRIORITY APPLIC. NO.: US 123791 APPLIC. DATE: 19990311

NATIONAL APPLIC. NO.: WO 2000US6676 APPLIC. DATE: 20000310

LANGUAGE: English

ABSTRACT: An array of peptide probes containing a porous membrane proximate to at least one electrode, is new. Also claimed are: producing an array of peptide probes for analyzing gene products; and making an array of one or more peptide probes for analyzing gene products. The methods can be used for determining the presence or quantiating the amount of a gene product in a biological sample. The array is also useful for identifying the functional binding domain of a gene product in a sample. The peptide probes arrays are useful for discovering new drug targets. (52pp)

DESCRIPTORS: gene product quantitation, peptide probe, electrode (Vol.20, No.2)

SECTION: GENETIC ENGINEERING AND FERMENTATION-Nucleic Acid Technology (A1)

3/5/69 (Item 1 from file: 399)

DIALOG(R) File 399: CA SEARCH(R)

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134219193 CA: 134(16)219193j CONFERENCE PROCEEDING

Miniaturized electrospraying as a technique for the production of microarrays of reproducible micrometer sized protein spots

AUTHOR(S): Moerman, Robert; Frank, Johannes; Marijnissen, Johannes C. M.; Schalkhamer, Thomas; van Dedem, Gijs W. K.

LOCATION: Kluyver Laboratory of Biotechnology, University of Technology, 2628 BC, Delft, Neth.

JOURNAL: Micro Total Anal. Syst. 2000, Proc. .mu.TAS Symp., 4th EDITOR:  
Van den Berg, Albert (Ed), Olthuis, W. (Ed), Bergveld, Piet (Ed), DATE:  
2000 PAGES: 557-560 CODEN: 69AJPB LANGUAGE: English PUBLISHER: Kluwer  
Academic Publishers, Dordrecht, Neth

SECTION:

CA209007 Biochemical Methods

IDENTIFIERS: miniaturized electrospraying microarray protein

DESCRIPTORS:

Electrodes...

counter; miniaturized electrospraying as a technique for prodn. of  
microarrays of reproducible micrometer sized protein spots

Spraying...

electrospraying; miniaturized electrospraying as a technique for prodn.  
of microarrays of reproducible micrometer sized protein spots

Capillary tubes... Drops... Electric field... Electrolytes... Meniscus...

Proteins, general, processes... Reagents... Surface electric charge...

Surfactants...

miniaturized electrospraying as a technique for prodn. of microarrays  
of reproducible micrometer sized protein spots

CAS REGISTRY NUMBERS:

56-81-5 107-21-1 uses, miniaturized electrospraying as a technique for  
prodn. of microarrays of reproducible micrometer sized protein spots

3/5/70 (Item 2 from file: 399)

DIALOG(R) File 399:CA SEARCH(R)

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134159600 CA: 134(12)159600c CONFERENCE PROCEEDING

Protein microarrays for monitoring of structural changes of proteins via  
surface enhanced metal nano cluster resonance

AUTHOR(S): Mayer, Christian; Palkovits, Roland; Bauer, Georg;  
Schalkhammer, Thomas

LOCATION: Kluyver L. for Biotechnology, TU-Delft, 2628BC, Delft, Neth.

JOURNAL: Micro Total Anal. Syst. 2000, Proc. .mu.TAS Symp., 4th EDITOR:  
Van den Berg, Albert (Ed), Olthuis, W. (Ed), Bergveld, Piet (Ed), DATE:  
2000 PAGES: 553-556 CODEN: 69AJPB LANGUAGE: English PUBLISHER: Kluwer  
Academic Publishers, Dordrecht, Neth

SECTION:

CA209001 Biochemical Methods

CA206XXX General Biochemistry

IDENTIFIERS: protein structure microarray surface enhanced resonance  
immobilization crosslinking

DESCRIPTORS:

Biosensors...

optical; protein microarrays for monitoring of structural changes of  
proteins via surface enhanced metal nano cluster resonance

Crosslinking... Immobilization, biochemical... Mirrors... pH... UV radiation

protein microarrays for monitoring of structural changes of proteins  
via surface enhanced metal nano cluster resonance

Conformation...

protein; protein microarrays for monitoring of structural changes of  
proteins via surface enhanced metal nano cluster resonance

Optical absorption...

resonance; protein microarrays for monitoring of structural changes of  
proteins via surface enhanced metal nano cluster resonance

Albumins, properties...

serum; protein microarrays for monitoring of structural changes of  
proteins via surface enhanced metal nano cluster resonance

CAS REGISTRY NUMBERS:

50-01-1 2718-90-3 3179-76-8 9001-37-0 9002-13-5 25087-17-6 protein  
microarrays for monitoring of structural changes of proteins via  
surface enhanced metal nano cluster resonance

7429-90-5 7440-06-4 7440-47-3 7440-57-5 uses, protein microarrays for  
monitoring of structural changes of proteins via surface enhanced metal  
nano cluster resonance

3/5/71 (Item 3 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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133307185 CA: 133(22)307185t JOURNAL  
Microarrays for the analysis of protein-ligand interactions  
AUTHOR(S): Gamer, Jurgen  
LOCATION: Graffinity Pharmaceutical Design GmbH, Heidelberg, Germany,  
JOURNAL: BIOSpektrum DATE: 2000 VOLUME: 6 NUMBER: 5 PAGES: 415-416  
CODEN: BOSPFD ISSN: 0947-0867 LANGUAGE: German PUBLISHER: Spektrum  
Akademischer Verlag  
SECTION:  
CA209002 Biochemical Methods  
IDENTIFIERS: microarray protein ligand interaction  
DESCRIPTORS:  
Proteins, specific or class...  
immobilized; microarrays for the anal. of protein-ligand interactions  
Proteins, specific or class...  
ligand-binding; microarrays for the anal. of protein-ligand  
interactions  
cDNA... Ligands...  
microarrays for the anal. of protein-ligand interactions

3/5/72 (Item 4 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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133293182 CA: 133(21)293182a PATENT  
Preparation of and methods for use of polypeptide microarrays  
INVENTOR(AUTHOR): Brown, Patrick; Haab, Brian  
LOCATION: USA  
ASSIGNEE: The Board of Trustees of the Leland Stanford Junior University  
PATENT: PCT International ; WO 200063701 A2 DATE: 20001026  
APPLICATION: WO 2000US10171 (20000414) \*US PV129449 (19990415)  
PAGES: 34 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: G01N-033/68A  
DESIGNATED COUNTRIES: CA; JP; US DESIGNATED REGIONAL: AT; BE; CH; CY; DE  
; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE  
SECTION:  
CA209001 Biochemical Methods  
IDENTIFIERS: microarray biochip polypeptide ligand antibody  
immobilization diagnosis  
DESCRIPTORS:  
Biotechnology...  
biochips; prepn. of and methods for use of polypeptide microarrays  
Dispensing apparatus...  
dosing; prepn. of and methods for use of polypeptide microarrays  
Analytical apparatus... Antibodies... Antigens... Blood analysis... Body  
fluid... Diagnosis... Fluorometry... Immobilization, biochemical...  
Immunoassay... Ligands... Peptides, uses...  
prepn. of and methods for use of polypeptide microarrays

3/5/73 (Item 5 from file: 399)  
DIALOG(R) File 399:CA SEARCH(R)  
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133219784 CA: 133(16)219784g PATENT  
Microarrays of peptide affinity probes for analyzing patterns of protein  
synthesis and their synthesis and methods for analyzing gene products  
INVENTOR(AUTHOR): Rossi, Francis M.; Montgomery, Donald D.  
LOCATION: USA  
ASSIGNEE: Combimatrix Corporation  
PATENT: PCT International ; WO 200053625 A2 DATE: 20000914  
APPLICATION: WO 2000US6676 (20000310) \*US PV123877 (19990311) \*US  
PV123791 (19990311)  
PAGES: 52 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C07K-001/00A  
DESIGNATED COUNTRIES: AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN;  
CR; CU; CZ; DE; DK; DM; DZ; EE; ES; FI; GE; GD; GE; GH; GM; HR; HU; ID; IL;

IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; US; UZ; VN; YU; ZW; AM; AZ; BY; BG; KB; KZ; MD; RU; TJ; TM  
DESIGNATED REGIONAL: GH; GM; KE; LS; MW; SD; SL; SZ; TZ; UG; ZW; AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA; GN; GW; ML; MR; NE; SN; TD; TG

SECTION:

CA209002 Biochemical Methods

CA203XXX Biochemical Genetics

CA234XXX Amino Acids, Peptides, and Proteins

IDENTIFIERS: peptide affinity probe proteome analysis bioelectrode

DESCRIPTORS:

Fluorescent dyes...

as reporter groups; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

Membrane electrodes...

bioelectrodes, peptide arrays immobilized on; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

Protective groups...

Fmoc, in synthesis of peptides on membrane electrodes; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

Electrochemistry...

for anal. of patterns of protein synthesis; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

Semiconductor devices...

for synthesis of peptide probe arrays; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

Antibodies...

in anal. of patterns of gene expression and of gene product ligand binding domain; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for

Peptide library...

ordered arrays; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

CAS REGISTRY NUMBERS:

76197-35-8 92557-80-7 as reporter group; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

68-19-9 1941-24-8 in prepn. of peptide arrays; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

58822-25-6P synthesis on membrane electrode of; microarrays of peptide affinity probes for analyzing patterns of protein synthesis and their synthesis and methods for analyzing gene products

3/5/74 (Item 1 from file: 420)

DIALOG(R)File 420:UnCover

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14072324 UnCover No.: 251130199197

New Protein Microarrays.

Chemical and engineering news. September 11, 2000, v. 78 n. 37, p. 6

ISSN: 0009-2347 JOURNAL CODE: 23182036

Devices could ease screening of cellular proteins en masse.

3/5/75 (Item 1 from file: 431)

DIALOG(R)File 431:MediConf: Medical Con. & Events

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00085975

REFERENCE NUMBER: 18943901

EVENT TITLE: Protein Microarray Technology

EVENT TYPE: Seminar  
DATES: from Wednesday, March 21, 2001 to Wednesday, March 21, 2001  
HOST SITE: The Westin Horton Plaza Hotel  
EVENT CITY: San Diego, California  
EVENT COUNTRY: United States/USA/Etats Unis/US/North America/Nord Amerika/  
Amerique du Nord  
COUNTRY CODE: C1906  
ORGANIZER: IBC USA Conferences, Inc.  
James Prudhomme, Life Sciences  
1 Research Drive, Suite 400A  
Westborough, MA 01581-5195, USA  
PHONE: +1 (508) 616-5550  
FAX: +1 (508) 616-5522  
E-MAIL: jprudhomme@ibcusa.com  
URL: http://www.ibcusa.com

DESCRIPTORS: pharmaceutical industry, pharmaceutical research,  
biotechnology, protein arrays, peptide arrays, rapid  
screening, rapid detection applications, high throughput  
screening, HTS

MEDICAL SPEC.: P8030 -- Pharmacy and Pharmacology/Pharmazie und  
Pharmakologie/Pharmacie et Pharmacologie  
P85121 -- Biochemistry and Biotechnology/Biochimie und  
Biotechnologie/Biochimie et Biotechnologie  
INPUT DATE: February 11, 2001

3/5/76 (Item 1 from file: 501)  
DIALOG(R) File 501:Extel Intl News Cards  
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00658866

Publication Date: September 12, 2000

Oxford GlycoSciences PLC  
Country: UNITED KINGDOM (GBR)

EXTTEL Company Number: 00038571  
SEDOL Number: 0264732  
Topic Code: OGS  
ISIN Number: GB0002647328

#### ACTIVITIES

Oxford GlycoSciences PLC  
Activities  
Company and Cambridge Antibody Technology ("CAT") announced collaboration  
to develop "protein chip" technology.  
Technology is for detection of **proteins** using antibody based **microarrays**

Companies will combine their respective technologies, to develop new  
**protein** detection and screening technology based on antibody **microarrays**  
. Company's human **protein** libraries and CAT's human antibody libraries  
will be analysed, paired **proteins** and antibodies selected and  
**microarrays** developed based on Company's current **protein microarray**  
prototype format. Goal is to create new generation of **protein** detection  
technology with speed, throughput and sensitivity to serve development of  
research tools, diagnostics and novel therapeutics. Each party will fund  
its own research contribution.

Section Heading(s): Activities

3/5/77 (Item 2 from file: 501)  
DIALOG(R) File 501:Extel Intl News Cards  
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00658865

Publication Date: September 19, 2000



Oxford GlycoSciences PLC  
Country: UNITED KINGDOM (GBR)

EXTEL Company Number: 00038571  
SEDOL Number: 0264732  
Topic Code: OGS  
ISIN Number: GB0002647328

#### ACTIVITIES

Oxford GlycoSciences PLC  
Activities

Company and Packard BioScience Company announced collaboration to apply respective technologies to develop protein biochips ("microarrays").

Section Heading(s): Activities

3/5/78 (Item 3 from file: 501)  
DIALOG(R) File 501:Extel Intl News Cards  
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00638481

Publication Date: September 12, 2000

Cambridge Antibody Technology Group PLC  
Country: UNITED KINGDOM (GBR)

EXTEL Company Number: 00037865  
SEDOL Number: 0166225  
Topic Code: CAT  
ISIN Number: GB0001662252

#### ACTIVITIES

Cambridge Antibody Technology Group PLC  
Activities

Company announced strategic research collaboration with Oxford GlycoSciences PLC.  
Research collaboration is to develop "Protein Chip" technology for detection of proteins using antibody based microarrays.  
Company and OGS will combine their respective world-leading technologies, to develop new protein detection and screening technology based on antibody microarrays. OGS's human protein libraries and Company's human antibody libraries will be analysed, paired proteins and antibodies selected and microarrays developed based on OGS's current protein microarray prototype format. Goal is to create new generation of protein detection technology, with speed, throughput and sensitivity to serve development of research tools, diagnostics and novel therapeutics. Each party will fund its own research contribution.

Section Heading(s): Activities

3/5/79 (Item 1 from file: 519)  
DIALOG(R) File 519:D&B-Duns Finan.Records Plus(TM)  
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For Full Record, choose Format 15 - Price = \$118.70

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3/5/80 (Item 1 from file: 660)  
DIALOG(R) File 660:Federal News Service  
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00246892 SUBFILE: Daybook  
GENERAL NEWS EVENTS - PART 2

MONDAY, NOVEMBER 15, 1999  
SECTION HEADING: Final Daily Schedule  
DATELINE: Washington dateline general news  
SCHEDULE DATE: 991115 YEAR: 1999

APPROXIMATE WORD COUNT: 000806 APPROXIMATE LINE COUNT: 00073  
FEDERAL NEWS SERVICE

For any additions or corrections to the Daybook, please call the  
Daybook editors, Joe Loomis or Pat Thorne at (202) 544-4812.

dest+ dbgen,dbcon,dbscitech,dbdefense

EVENT: WORKSHOPS - NATIONAL ACADEMY OF SCIENCES (NAS) COMMISSION  
ON ENGINEERING AND TECHNICAL SYSTEMS DIVISION ON MILITARY  
SCIENCE AND TECHNOLOGY

SUBJECT: National Academy of Sciences (NAS) Commission on  
Engineering and technical Systems Division on Military  
Science and Technology holds a workshop on "Alternative  
Technologies to Replace Anti-Personnel Landmines."  
(November 15-16)

Highlights:

- 9 am - Closed Session
- 1:30 pm - George Bugliarello, chairman, Committee on  
Alternative Technologies to Replace Anti-Personnel  
Landmines, Welcoming Remarks
- 1:45 pm - "Staffing for Study"
- 1:50 pm - "Administrative Procedures"
- 2 pm - "background for Study"
- 3 pm - "Review Statement of Task"
- 3:30 pm - "Strategic and Tactical Landmine Usage  
Overview"
- 5 pm - Committee Organization/Chairman's Time

LOCATION: Sheraton National Hotel, Columbia Pike and Washington  
Boulevard, Arlington, VA  
-- November 15, 1999

CONTACT: Christina Maiers, 202-334-2644 or <http://www.nas.edu>

dest+ dbgen,dbcon,dbhlth,dbmedi

EVENT: CONFERENCE - CAMBRIDGE HEALTHTECH INSTITUTE (CHI)

SUBJECT: Cambridge Healthtech Institute (CHI) holds a conference on  
"Protein Structure." (November 15-16)

Highlights:

- 8:30 am - Dr. Min S. Parker, technical staff member,  
Los Alamos National Laboratory, Opening Remarks
- 8:40 am - Dr. Wayne A. Hendrickson, biochemistry  
professor, Howard Hughes Medical Institute (HHMI) Research  
Laboratories, and biochemistry and molecular biophysics  
department, Columbia University, Keynote Address:  
"Structure Methods Becoming Structural Genomics"
- 9:15 am - "Structural Proteomics: A method of Target  
Validation and Invalidation"
- 10:15 am - "Structural genomics and Automated Structure  
Selection"
- 10:45 am - "The Role of NMR in Structural Genomics"
- 11:15 am - Panel Discussions
- 1:30 pm - Dr. John Moult, computational biology  
professor, Center for Advanced Research in Biotechnology,  
University of Maryland Biotechnology Institute
- 1:35 pm - "Computational Approaches to Structural

Genomics"

- 2:05 pm - "Large-Scale Comparative Protein Modeling"
- 2:35 pm - "Comparative Protein Structure Modeling in Genomics"
- 3:45 pm - "Proteome Databases for Applications of Model Organism Knowledge to Nonmodel Organisms"
- 4:15 pm - "Structure-Based Assignment of Molecular Functions of Hypothetical Proteins"
- 4:45 pm - "Deriving Function From Protein Structure Modeling"
- 5:15 pm - Panel Discussion

LOCATION: Capital Hilton Hotel, 16th and K Streets NW, Washington, DC

-- November 15, 1999

CONTACT: Jennifer Laakso, 617-630-1385 or  
<http://www.healthtech.com/conference/pst/pst.htm>

-----  
dest+ dbgen,dbcon,dbhlth,dbmedi,dbscitech

EVENT: CONFERENCE - CAMBRIDGE HEALTHTECH INSTITUTE (CHI)

SUBJECT: Cambridge Healthtech Institute (CHI) holds a conference on "Chemokine and Chemokine Receptors: Disease Targets for Therapeutic Development." (November 15-16)

Highlights:

- 9 am - Dr. Thomas J. Schall, president and CEO, Chemocentryx Inc., Opening Comments
- 9:10 am - "Chemokines at the dawn of the 21st Century: Aliens and Archetypes"
- 9:50 am - "The ELR-CXC-Chemokine Platelet Basic Protein (PBP) Potently Desensitizes Chemokine-Induced Neutrophil Activation"
- 10:45 am - "Changes in Chemokine Receptor Expression Patterns During T Cell Development"
- 11:15 am - "CCR4 Knockout Mouse Models of Lung Inflammation"
- 11:45 pm - Panel Discussion
- 1:40 pm - Dr. Joseph Hesselgesser, research scientist, Immunology, Berlex BioSciences
- 1:45 pm - "Mechanisms of Leukocyte Trafficking Across the Blood Brain Barrier"
- 2:15 pm - "Chemokines and Their Receptors in Multiple Sclerosis"
- 2:45 pm - "Chemokine and Chemokine Receptor Expression Patterns Regulate the Pathogenesis of Autoimmune Encephalomyelitis"
- 3:45 pm - "Inhibition of Angiogenesis Induced by IP-10/CRG-2"
- 4:15 pm - "Chemokines and Their Receptors in Allograft Rejection"
- 4:45 pm - Panel Discussion

LOCATION: Ritz Carlton Tysons Corner Hotel, 1700 Tysons Boulevard, McLean, VA

-- November 15, 1999

CONTACT: Jennifer Laakso, 617-630-1385 or  
<http://www.healthtech.com/conference/kmo/kmo.htm>

-----  
dest+ dbgen,dbcon,dbdepen,dbscitech

EVENT: CONFERENCE - ENERGY DEPARTMENT OAK RIDGE NATIONAL LABORATORY (ORNL) ENVIRONMENTAL SCIENCES DIVISION

SUBJECT: Energy Department Oak Ridge National Laboratory (ORNL)

Environmental Sciences Division holds its "Seventh Annual Conference on Small Genomes." (November 14-17)

Highlights:

- 8 am - "Functional Analysis of the Vibrio Cholerae Genome"
- 8:45 am - "Printing Technologies for Genomic Microarrays"
- 9:15 am - "Advances in Microarray Scanning and Analysis"
- 10 am - "Flowthrough Geosensor Chips and Their Application in Microbial Genomics"
- 10:30 am - "High Throughput, Low Cost Oligonucleotides for Gene Expression Analysis"
- 11:30 am - "Whole-Genome RNA and Protein Regulatory Networks in E. Coli and S. Cerevisiae"
- 1 pm - "Gene Transfer Systems for Extremophiles"
- 1:30 pm - "Essential and Dispensable Genes in Mycoplasmas"
- 2:30 pm - "Rapid Identification and Precise Expression Profiling of Proteomes by Mass Spectrometry"
- 3:15 pm - "Exploration of Genome Usage in Methanococcus Jannaschii and Pyrococcus Furiosus From the Protein Point-of-View"
- 4:15 pm - "Using Genomics Databases to Understand Protein Expression Profiles of M. Tuberculosis"
- 5 pm - Poster Session

LOCATION: Doubletree Hotel, 300 Army Navy Drive, Arlington, VA  
-- November 15, 1999

CONTACT: Kim Smith, 423-576-4860 or  
<http://www.esd.ornl.gov/programs/microbes/agenda2.html>

KEYWORDS: daybook; gen; II; mon

DAYBOOK DESCRIPTORS: General News Events, Conventions, conferences, symposiums, forums, Science and technology, Defense; General News Events, Conventions, conferences, symposiums, forums, Health, Medicine (technology); General News Events, Conventions, conferences, symposiums, forums, Health, Medicine (technology), Science and technology; General News Events, Conventions, conferences, symposiums, forums, Dept. of Energy, DOE, Science and technology

?logoff

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30apr01 16:42:32 User026066 Session D6423.6
Sub account: 3776-010140-BEJ-LAUNCHCYTE
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$0.04 Estimated cost File2
$0.06      0.011 DialUnits File5
$0.06 Estimated cost File5
$0.07      0.011 DialUnits File8
$0.07 Estimated cost File8
$0.04      0.007 DialUnits File16
$0.04 Estimated cost File16
$0.01      0.011 DialUnits File20
$0.01 Estimated cost File20
$0.41      0.029 DialUnits File34
$8.40      2 Type(s) in Format 5
$8.40      2 Types
$8.81 Estimated cost File34
$0.08      0.011 DialUnits File71
$0.08 Estimated cost File71
$0.12      0.014 DialUnits File73
$2.35      1 Type(s) in Format 5
$2.35      1 Types
$2.47 Estimated cost File73
$0.06      0.011 DialUnits File76
$0.06 Estimated cost File76
$0.03      0.007 DialUnits File88
$0.03 Estimated cost File88
$0.04      0.011 DialUnits File94
```

### Status: Path 1 of [Dialog]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID dialog.com)  
Trying 3106...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 00.12.12D

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Logon file001 18apr01 15:28:33

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Set Items Description

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18apr01 15:33:23 User026066 Session D6404.1

Sub account: 2087-010140-LAUNCHCYTE-BEJ

\$0.20 0.057 DialUnits File1

\$0.20 Estimated cost File1

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\$1.20 Estimated cost this search

\$1.20 Estimated total session cost 0.057 DialUnits

File 349:PCT Fulltext 1983-2001/UB=20010412, UT=20010329

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Set Items Description

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E2	1	PA=ZYO YASUSHI
E3	3	*PA=ZYOMYX
E4	3	PA=ZYOMYX INC
E5	1	PA=ZYPMAN
E6	1	PA=ZYPMAN FREDY R
E7	1	PA=ZYQAD
E8	1	PA=ZYQAD LIMITED
E9	3	PA=ZYRA
E10	3	PA=ZYRA STAN
E11	1	PA=ZYRYANOV
E12	1	PA=ZYRYANOV VIKTOR YAKOVLEVICH

Enter P or PAGE for more

?s e3-e4

3 PA=ZYOMYX  
3 PA=ZYOMYX INC  
3 E3-E4

S1

?t 1/5/1

1/5/1

DIALOG(R)File 349:PCT Fulltext

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00691492

**MICRODEVICES FOR SCREENING BIOMOLECULES**

**MICRODISPOSITIFS SERVANT A CRIbler DES BIOMOLECULES**

Patent Applicant/Assignee:

**ZYOMYX INC, ZYOMYX, INC.**, 3912 Trust Way, Hayward, CA 94545, US

Inventor(s):

WAGNER Peter, WAGNER, Peter, 2211 Village Court &7, Belmont, CA 94002, US  
AULT-RICHE Dana, AULT-RICHE, Dana, 972 Cajon Way, Palo Alto, CA 94303, US  
NOCK Steffen, NOCK, Steffen, 3629 Glenwood Avenue, Redwood City, CA  
94062, US

ITIN Christian, ITIN, Christian, 315 Waverley Street &3, Menlo Park, CA  
94025, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0004390 A2 20000127 (WO 200004390)

Application: WO 99US15969 19990714 (PCT/WO US9915969)

Priority Application: US 98115397 19980714

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE

DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR

KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI

SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW

AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC

NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: G01N-033/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 21176

English Abstract

Methods and devices for the parallel, *in vitro* screening of biomolecular activity using miniaturized microfabricated devices are provided. The biomolecules immobilized on the surface of the devices of the present invention include proteins, polypeptides, polynucleotides, polysaccharides, phospholipids, and related unnatural polymers of biological relevance. These devices are useful in drug development, functional proteomics and clinical diagnostics and are preferably used for the parallel screening of families of related proteins.

French Abstract

L'invention concerne des procedes et des dispositifs permettant de mettre en oeuvre un criblage *in vitro* en parallele d'activite biomoleculaire au moyen de dispositifs microfabriques miniaturises. Les biomolecules immobilisees a la surface des dispositifs de la presente invention comprennent des proteines, des polypeptides, des polynucleotides, des polysaccharides, des phospholipides et des polymeres non naturels apparentes utiles en biologie. Ces dispositifs sont utiles dans le developpement de medicaments, la proteomique fonctionnelle et le diagnostic clinique, et sont de preference utilises pour cribler en parallele des familles de proteines apparentees.

?logoff hold

18apr01 15:34:16 User026066 Session D6404.2

Sub account: 2087-010140-LAUNCHCYTE-BEJ

\$1.09 0.229 DialUnits File349

\$5.10 1 Type(s) in Format 5

\$5.10 1 Types

\$6.19 Estimated cost File349

\$0.19 TELNET

\$6.38 Estimated cost this search  
\$7.58 Estimated total session cost 0.286 DialUnits

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### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID dialog.com)  
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File 349:PCT Fulltext 1983-2001/UB=20010412, UT=20010329

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Set Items Description

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Terminal set to DLINK

?t 1/5/2-3

1/5/2

DIALOG(R)File 349:PCT Fulltext

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00691491

ARRAYS OF PROTEIN-CAPTURE AGENTS AND METHODS OF USE THEREOF

GROUPEMENTS D'AGENTS D'INTERCEPTION DE PROTEINE ET PROCEDES D'UTILISATION  
DE CEUX-CI

Patent Applicant/Assignee:

ZYOMYX INC, ZYOMYX, INC., 3912 Trust Way, Hayward, CA 94545, US

Inventor(s):

WAGNER Peter, WAGNER, Peter, 2211 Village Court &7, Belmont, CA 94002, US

NOCK Steffen, NOCK, Steffen, 3629 Glenwood Avenue, Redwood City, CA

94062, US

AULT-RICHE Dana, AULT-RICHE, Dana, 972 Cajon Way, Palo Alto, CA 94303, US

ITIN Christian, ITIN, Christian, 315 Waverley Street &3, Menlo Park, CA

94025, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0004389 A2 20000127 (WO 200004389)

Application: WO 99US15968 19990714 (PCT/WO US9915968)

Priority Application: US 98115455 19980714

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ CZ DE

DE DK DK EE EE ES FI FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR

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AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC  
NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: G01N-033/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 26401

#### English Abstract

Arrays of protein-capture agents useful for the simultaneous detection of a plurality of proteins which are the expression products, or fragments thereof, of a cell or population of cells in an organism are provided. A variety of antibody arrays, in particular, are described. Methods of both making and using the arrays of protein-capture agents are also disclosed. The invention arrays are particularly useful for various proteomics applications including assessing patterns of protein expression and modification in cells.

#### French Abstract

L'invention concerne des groupements d'agents d'interception de proteine utiles pour mettre en oeuvre une detection simultanee de plusieurs proteines qui sont les produits d'expression, ou des fragments de ceux-ci, d'une cellule ou d'une population de cellules dans un organisme. Divers groupements d'anticorps sont notamment decrits. L'invention concerne egalement des procedes de fabrication et d'utilisation des groupements d'agents d'interception de proteine. Les groupements de l'invention sont particulierement utiles pour diverses applications de proteomique, y compris pour evaluer des motifs d'expression et de modification de proteine dans des cellules.

1/5/3

DIALOG(R) File 349: PCT Fulltext

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00691485 \*\*Image available\*\*

#### ARRAYS OF PROTEINS AND METHODS OF USE THEREOF

#### GROUPEMENTS DE PROTEINES ET PROCEDES D'UTILISATION DE CEUX-CI

Patent Applicant/Assignee:

ZYOMYX INC, 3912 Trust Way, Hayward, CA 94545, US, US (Residence), US  
(Nationality)

Inventor(s):

WAGNER Peter, 2211 Village Court #7, Belmont, CA 94002, US,  
AULT-RICHE Dana, 972 Cajon Way, Palo Alto, CA 94303, US,  
NOCK Steffen, 3629 Glenwood Avenue, Redwood City, CA 94062, US,  
ITIN Christian, 315 Waverley Street #3, Menlo Park, CA 94025, US,

Legal Representative:

CHOW Y Ping (et al) (agent), Heller Ehrman White & McAuliffe, 525  
University Avenue, Palo Alto, CA 94301-1900, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200004382 A1 20000127 (WO 0004382)

Application: WO 99US15971 19990714 (PCT/WO US9915971)

Priority Application: US 98115455 19980714

Designated States: AE AL AM AT AT (utility model) AU AZ BA BB BG BR BY CA  
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UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-033/543

International Patent Class: G01N-033/551

Publication Language: English

Filing Language: English



Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 20640

#### English Abstract

Protein arrays for the parallel, in vitro screening of biomolecular activity are provided. Methods of using the protein arrays are also disclosed. On the arrays, a plurality of different proteins, such as different members of a single protein family, are immobilized on one or more organic thin films on the substrate surface. The protein arrays are particularly useful in drug development, proteomics, and clinical diagnostics.

#### French Abstract

L'invention concerne des groupements de proteines permettant de mettre en oeuvre un criblage in vitro en parallele d'activite biomoleculaire. Des procedes d'utilisation des groupements de proteines sont egalement decrits. Dans les groupements, plusieurs proteines differentes telles que des membres differents d'une seule famille de proteines, sont immobilisees sur un ou plusieurs films minces organiques a la surface du substrat. Les groupements de proteines sont particulierement utiles dans le developpement de medicaments, la proteomique et le diagnostic clinique.

#### Legal Status (Type, Date, Text)

Correction 20010315 Corrections of entry in Section 1: under (81) add  
"AT (utility model), CZ (utility model, DE (utility  
model), DK (utility model), EE (utility model), FI  
(utility model), SK (utility model)"

Republication 20010315 A1 With international search report.  
?e pa=ciphergen?

Ref	Items	Index-term
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E4	4	PA=CIPHERIT
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E12	1	PA=CIPKUS LORETTA A

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S2 10 E1-E2  
?t 2/5/1-10

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00792304 \*\*Image available\*\*  
**PROSTATE CANCER MARKER PROTEINS**  
**PROTEINES MARQUEUR DU CANCER DE LA PROSTATE**  
Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, 6611 Dumbarton Circle, Fremont, CA 94555, US,  
US (Residence), US (Nationality), (For all designated states except:  
US:

EASTERN VIRGINIA MEDICAL SCHOOL, 700 West Olney Road, Norfolk, VA 23507,  
US, US (Residence), US (Nationality), (For all designated states  
except: US

Patent Applicant/Inventor:

YIP Tai-Tung, 7515 Kingbury Court, Cupertino, CA 95014, US, US  
(Residence), US (Nationality), (Designated only for: US)

YIP Christine, 7515 Kingbury Court, Cupertino, CA 95014, US, US  
(Residence), US (Nationality), (Designated only for: US)

WRIGHT George L Jr, 829 Moultrie Court, Virginia Beach, VA 23455, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

CHOI Kathleen L (et al) (agent), Townsend and Townsend and Crew LLP, Two  
Embarcadero Center, 8th Floor, San Francisco, CA 94111-3834, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200125791 A2 20010412 (WO 0125791)

Application: WO 2000US27682 20001006 (PCT/WO US0027682)

Priority Application: US 99158422 19991007

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ

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(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-033/574

Publication Language: English

Filing Language: English

English Abstract

The invention provides methods and kits that can be used as an aid for  
prostate cancer diagnosis using markers that are differentially present  
in the samples of prostate cancer patients and subjects who do not have  
prostate cancer (e.g., benign prostate hyperplasia patients or normal,  
healthy subjects).

French Abstract

L'invention concerne des techniques et des kits contribuant a  
diagnostiquer le cancer de la prostate a l'aide de marqueurs qui sont  
présents dans les échantillons des patients souffrant du cancer de la  
prostate et des sujets qui ne sont pas atteints du cancer de la prostate  
(p. ex. patients souffrant d'hyperplasie prostatique benigne ou des  
sujets normaux et en bonne sante).

Legal Status (Type, Date, Text)

Publication 20010412 A2 Without international search report and to be  
republished upon receipt of that report.

2/5/2

DIALOG(R) File 349:PCT Fulltext

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00757250 \*\*Image available\*\*

VARIABLE WIDTH DIGITAL FILTER FOR TIME-OF-FLIGHT MASS SPECTROMETRY

FILTRE NUMERIQUE A LARGEUR VARIABLE POUR SPECTROMETRIE DE MASSE DE TEMPS DE  
VOL

Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, 490 San Antonio Road, Palo Alto, CA

94306-4636, US, US (Residence), US (Nationality), (For all designated  
states except: US

Patent Applicant/Inventor:

GAVIN Edward J, 1440 Isabella Street, Santa Clara, CA 95050, US, US

(Residence), US (Nationality), (Designated only for: US)

BRAGINSKY Leonid, 107 Hagen Road, Newton, MA 02459, US, US (Residence),

US (Nationality), (Designated only for: US)

Legal Representative:

LEMOND Kevin T, Townsend and Townsend and Crew LLP, Two Embarcadero

Center, Eighth Floor, San Francisco, CA 94111-3834, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200070648 A2 20001123 (WO 0070648)  
Application: WO 2000US13153 20000512 (PCT/WO US0013153)  
Priority Application: US 99134072 19990513  
Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE  
DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC  
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SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM  
Main International Patent Class: H01J-049/40  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 2978

#### English Abstract

A method and system of detecting mass to charge ratio of ions. The method includes producing charged ions in a vacuum, accelerating the charged ions in an electric field into a free flight tube and detecting the charged ions at a detector associated with the free flight tube. A control system selects a bandwidth for filtering a signal produced by the detector and the signal produced by the detector is then filtered with a variable width digital filter based upon the selected bandwidth. The bandwidth for filtering the signal may be selected from a look-up table within the control system based upon the mass to charge ratio of an ion of interest. Alternatively, a peak bandwidth within the signal produced by the detector may be determined and the signal produced by the detector may then be filtered with the variable width digital filter based upon the determined peak bandwidth.

#### French Abstract

L'invention concerne un procede et un systeme de detection du rapport masse-charge d'ions. Le procede consiste a introduire des ions charges dans un vide, a accelerer les ions charges dans un champ electrique dans un tube de vol libre puis a detecter les ions charges dans un detecteur associe au tube de vol libre. Un systeme de commande selectionne une largeur de bande de filtrage d'un signal produit par le detecteur puis le signal produit par le detecteur est filtre avec un filtre numerique a largeur variable sur la base de la largeur de bande selectionnee. La largeur de bande de filtrage du signal peut etre selectionnee dans une table de recherche dans le systeme de commande sur la base du rapport masse-charge d'un ion interessant. Dans un autre mode de realisation, on peut determiner une largeur de bande de crete dans le signal produit par le detecteur et on peut filtrer le signal produit par le detecteur avec le filtre numerique a largeur variable sur la base de la largeur de bande de crete determinee.

#### Legal Status (Type, Date, Text)

Publication 20001123 A2 Without international search report and to be republished upon receipt of that report.  
Search Rpt 20010201 Late publication of international search report  
Examination 20010308 Request for preliminary examination prior to end of 19th month from priority date

2/5/3

DIALOG(R)File 349:PCT Fulltext  
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00757249 \*\*Image available\*\*

OPTICAL BENCH FOR LASER DESORPTION/IONIZATION MASS SPECTROMETRY  
BANC OPTIQUE POUR SPECTROMETRIE DE MASSE PAR DESORPTION/IONISATION LASER  
Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, 490 San Antonio Road, Palo Alto, CA  
94306-4636, US, US (Residence), US (Nationality), (For all designated

states except: US  
Patent Applicant/Inventor:  
WEINBERGER Scot R, 657 George Street, Montara, CA 94307, US, US  
(Residence), US (Nationality), (Designated only for: US)  
BRYAN Raymond G, 173 Nottingham Ct., Reno, NV 89511, US, US (Residence),  
US (Nationality), (Designated only for: US)  
Legal Representative:  
LEMOND Kevin T, Townsend Townsend and Crew LLP, Two Embarcadero Center,  
8th floor, San Francisco, CA 94111-3834, US  
Patent and Priority Information (Country, Number, Date):  
Patent: WO 200070647 A1 20001123 (WO 0070647)  
Application: WO 2000US12984 20000512 (PCT/WO US0012984)  
Priority Application: US 99134071 19990513  
Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE  
DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC  
LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK  
SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM  
Main International Patent Class: H01J-049/16  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 5667

#### English Abstract

A laser optical bench for use with a laser desorption/ionization mass spectrometer. The laser optical bench includes a laser for producing light, a focusing structure that receives light from the laser and focuses predominantly in a single plane, an attenuator that receives light from the focusing structure, beam steering structure for directing light from the attenuator from the target; and a final focusing element for focusing light from the beam steering structure on the target. Further focusing elements may be included for further focusing and dispersing the light beam in different planes. Additionally, photodetectors or photodiodes may be included for energy measurement and sensing a lasing event.

#### French Abstract

L'invention concerne un banc optique laser qui s'utilise avec un spectrometre de masse par desorption/ionisation laser. Le banc optique laser inclut un laser qui emit de la lumiere, une structure de focalisation qui recoit la lumiere du laser et focalise principalement sur un seul plan, un affaiblisseur qui recoit la lumiere de la structure de focalisation, une structure de pointage du faisceau qui oriente la lumiere de l'affaiblisseur vers une cible, et un element de focalisation terminal qui focalise la lumiere de la structure de pointage du faisceau sur la cible. D'autres elements de focalisation peuvent etre inclus pour effectuer d'autres focalisations et disperser le faisceau lumineux sur differents plans. En outre, des photodetecteurs et des photodiodes peuvent etre inclus pour mesurer l'energie et detecter un evenement de lasage.

#### Legal Status (Type, Date, Text)

Publication 20001123 A1 With international search report.  
Publication 20001123 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.  
Examination 20010315 Request for preliminary examination prior to end of 19th month from priority date

2/5/4

DIALOG(R) File 349:PCT Fulltext,  
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00754266

**PROBES FOR A GAS PHASE ION SPECTROMETER  
SONDES POUR SPECTROMETRE D'IONS EN PHASE GAZEUSE**

Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, 490 San Antonio Road, Palo Alto, CA  
94306-4636, US, US (Residence), US (Nationality), (For all designated  
states except: US

Patent Applicant/Inventor:

RICH William E, 840 Corriente Point Drive, Redwood Shores, CA 94565, US,  
US (Residence), US (Nationality), (Designated only for: US)  
UM Pil-Je, 385 Mountain View Drive # 4, Daly City, CA 94014, US, US  
(Residence), KR (Nationality), (Designated only for: US)  
VOIVODOV Kamen, 3382 Pine Wood Court, Hayward, CA 94542, US, US  
(Residence), US (Nationality), (Designated only for: US)  
YIP Tai-Tung, 7515 Kingsbury Court, Cupertino, CA 95014, US, US  
(Residence), -- (Nationality), (Designated only for: US)  
BEECHER Jody, 1919 Kirkland Avenue, San Jose, CA 95125, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

WEBER Kenneth (et al) (agent), Townsend and Townsend and Crew LLP, 8th  
Floor, Two Embarcadero Center, San Francisco, CA 94111, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200066265 A2 20001109 (WO 0066265)

Application: WO 2000US11452 20000427 (PCT/WO US0011452)

Priority Application: US 99560715 19990427; US 99131652 19990429

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE

DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC

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(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: B01L

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 17966

**English Abstract**

The invention provides a probe and a method of making the probe that is removably insertable into a gas phase ion spectrometer, the probe comprising a substrate having a surface and a hydrogel material on the surface, the hydrogel material comprising binding functionalities for binding with an analyte detectable by the gas phase ion spectrometer. The invention also provides a probe and a method of making the probe that is removably insertable into a gas phase ion spectrometer, the probe comprising a substrate having a surface and a plurality of particles that are uniform in diameter on the surface, the particles comprising binding functionalities for binding with an analyte detectable by the gas phase ion spectrometer. Further, the invention provides a system comprising the probe of the present invention and a gas phase ion spectrometer comprising an energy source that directs light to the probe surface to desorb an analyte and a detector in communication with the probe surface that detects the desorbed analyte. The invention also provides a method for desorbing an analyte from a probe surface, the method comprising exposing the binding functionalities to a sample containing an analyte under conditions to allow binding between the analyte and the binding functionalities, and desorbing the analyte from the probe by gas phase ion spectrometry.

**French Abstract**

L'invention concerne une sonde et un procede de fabrication de cette sonde, laquelle peut etre inseree de facon amovible dans un spectrometre d'ions en phase gazeuse. Ladite sonde comprend un substrat presentant une surface et une matiere hydrogel deposee sur ladite surface. Ladite

matiere presente des fonctions de liaison qui lui permettent de se lier avec la substance a analyser, laquelle substance peut etre detectee au moyen d'un spectrometre d'ions en phase gazeuse. L'invention concerne egalement une sonde et un procede de fabrication de cette sonde, laquelle peut etre inseree de facon amovible dans un spectrometre d'ions en phase gazeuse. Ladite sonde comprend un substrat presentant une surface et une pluralite de particules de diametre identique deposees sur ladite surface. Lesdites particules presentent des fonctions de liaison qui leur permettent de se lier avec la substance a analyser, laquelle substance peut etre detectee au moyen d'un spectrometre d'ions en phase gazeuse. En outre, l'invention concerne un dispositif comprenant une telle sonde, un spectrometre d'ions en phase gazeuse comprenant une source d'energie qui dirige la lumiere vers la surface de ladite sonde afin de desorber la substance a analyser ; et un detecteur, mis en contact avec la surface de ladite sonde, qui detecte la substance a analyser ainsi desorbee. L'invention concerne egalement un procede de desorption d'une substance a analyser provenant d'une surface de la sonde. Ladite methode consiste a mettre les fonctions de liaison en contact avec un echantillon contenant une substance a analyser dans des conditions permettant la liaison entre ladite substance et lesdites fonctions de liaison et a desorber, par spectrometrie d'ions en phase gazeuse, la substance a analyser provenant de la sonde.

Legal Status (Type, Date, Text)

Publication 20001109 A2 Without international search report and to be republished upon receipt of that report.  
Examination 20001221 Request for preliminary examination prior to end of 19th month from priority date  
Correction 20010315 Corrections of entry in Section 1: under (30) replace "Not furnished, 27 April 2000 (27.04.00), US" by "09/560,715, 27 April 1999 (27.04.99), US" and under (72, 75) replace "VOIVODOV, Kamen [BG/US]" by "VOIVODOV, Kamen [US/US]"  
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2/5/5

DIALOG(R) File 349:PCT Fulltext

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00753906 \*\*Image available\*\*

**SAMPLE HOLDER WITH HYDROPHOBIC COATING FOR GAS PHASE MASS SPECTROMETERS  
SUPPORT D'ECHANTILLONS A REVETEMENT HYDROPHOBE POUR SPECTROMETRE DE MASSE  
EN PHASE GAZEUSE**

Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, Suite B, 490 San Antonio Road, Palo Alto, CA 94306, US, US (Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

BEECHER Jody, 1919 Kirkland Avenue, San Jose, CA 95125, US, US (Residence), US (Nationality), (Designated only for: US)

SCHEUFELE Frank, 809 Northampton Drive, Palo Alto, CA 94303, US, US (Residence), US (Nationality), (Designated only for: US)

VOIVODOV Kamen, 3382 Pine Wood Court, Hayward, CA 94542, US, US (Residence), BG (Nationality), (Designated only for: US)

WEINBERGER Scot, 657 George Street, Montara, CA 94307, US, US (Residence), US (Nationality), (Designated only for: US)

LANDGRAF William C, 762 Stone Lane, Palo Alto, CA 94303, US, US (Residence), US (Nationality), (Designated only for: US)

Legal Representative:

WEBER Kenneth A, Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200067293 A1 20001109 (WO 0067293)

Application: WO 2000US11499 20000427 (PCT/WO US0011499)

Priority Application: US 99131653 19990429

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KZ LC

LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK  
SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW  
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Main International Patent Class: H01J-049/04

International Patent Class: G01N-035/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 4421

English Abstract

This invention provides sample holder for mass spectrometry including a substrate having a surface and a film that coats the surface. The film includes openings that define features for the presentation of an analyte. The film also has a lower surface tension than the surface tension of the substrate surface, and has a water contact angle between 120degrees and 180degrees.

French Abstract

L'invention porte sur un support d'échantillons pour spectromètre de masse comportant un substrat dont la surface est revêtue d'un film, comportant des ouvertures aux caractéristiques permettant la présentation d'un analyte, dont la tension superficielle est inférieure à celle de la surface du substrat, et dont l'angle de raccordement de l'eau est compris entre 120degrees et 180degrees.

Legal Status (Type, Date, Text)

Publication 20001109 A1 With international search report.

Publication 20001109 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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Withdrawal 20010111 Withdrawal of priority claims after international publication: US Not furnished/non communiqué  
20000427

2/5/6

DIALOG(R)File 349:PCT Fulltext

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00632891 \*\*Image available\*\*

**SECONDARY ION GENERATOR DETECTOR FOR TIME-OF-FLIGHT MASS SPECTROMETRY**  
**DETECTEUR COMPRENANT UN GENERATEUR D'IONS SECONDAIRES POUR SPECTROMETRIE DE**  
**MASSE A TEMPS DE VOL**

Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, CIPHERGEN BIOSYSTEMS, INC. , 490 San Antonio  
Road, Palo Alto, CA 94306-4636 , US

Inventor(s):

WEINBERGER Scot R, WEINBERGER, Scot, R. , 657 George Street, Montara, CA  
94307 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9916103 A1 19990401

Application: WO 98US19865 19980922 (PCT/WO US9819865)

Priority Application: US 9759828 19970923

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV  
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG  
US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT  
BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA  
GN GW ML MR NE SN TD TG

Main International Patent Class: H01J-049/02;

International Patent Class: H01J-049/40;

Publication Language: English

Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 9739

#### English Abstract

An ion detector includes a secondary charged particle generator that generates secondary charged particles in response to primary ions that engage the secondary charged particle generator. The secondary charged particle generator has an electrostatic potential that repels the secondary charged particles toward an electro-emissive detector that generates electrons in response to primary ions and secondary charged particles that engage the electro-emissive detector. The electro-emissive detector has a field that attracts the secondary charged particles. An anode is provided for detecting electrons generated by the electro-emissive detector and for generating a signal.

#### French Abstract

Un detecteur d'ions comprend un generateur de particules chargees secondaires qui genere des particules chargees secondaires en reponse a des ions primaires qui entrent dans le generateur de particules chargees secondaires. Le generateur de particules chargees secondaires comprend un potentiel electrostatique qui repousse les particules chargees secondaires vers un detecteur emettant des electrons qui genere des electrons en reponse aux ions primaires et aux particules chargees secondaires qui entrent dans le detecteur emettant des electrons. Ce detecteur emettant des electrons comporte un champ qui attire les particules chargees secondaires. Une anode est prevue pour detecter les electrons generes par le detecteur emettant des electrons et pour generer un signal.

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DIALOG(R) File 349:PCT Fulltext  
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00613770      \*\*Image available\*\*  
RETENTATE CHROMATOGRAPHY AND PROTEIN CHIP ARRAYS WITH APPLICATIONS IN  
BIOLOGY AND MEDICINE  
CHROMATOGRAPHIE SUR PRODUIT RETENU ET ARRANGEMENTS DE PROTEINES SUR CIRCUIT  
AVEC APPLICATIONS BIOLOGIQUES ET MEDICALES

#### Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, CIPHERGEN BIOSYSTEMS, INC. , Suite B, 470 San  
Antonio Road, Palo Alto, CA 94306 , US

#### Inventor(s):

HUTCHENS T William, HUTCHENS, T., William , 28 Carriage Court, Los Altos,  
CA 95618 , US  
YIP Tai-Tung, YIP, Tai-Tung , 7515 Kingsbury Court, Cupertino, CA 95014 ,  
US

#### Patent and Priority Information (Country, Number, Date):

Patent: WO 9859362 A1 19981230  
Application: WO 98US12908 19980619 (PCT/WO US9812908)  
Priority Application: US 9754333 19970620; US 9767484 19971201

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
ML MR NE SN TD TG

Main International Patent Class: H01J-049/04;

Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 38809

#### English Abstract



This invention provides methods of retentate chromatography for resolving analytes in a sample. The methods involve adsorbing the analytes to a substrate under a plurality of different selectivity conditions, and detecting the analytes retained on the substrate by desorption spectrometry. The methods are useful in biology and medicine, including clinical diagnostics and drug discovery.

#### French Abstract

L'invention concerne des procedes de chromatographie sur produit retenu destines a separer des analytes dans un echantillon. Ces procedes consistent tout d'abord a adsorber lesdits analytes sur un substrat, dans des conditions de selectivite variable, puis a detecter les analytes accumules sur ce substrat par spectrometrie de desorption. Ces procedes sont utiles en biologie et en medecine, notamment pour des diagnostics cliniques et la recherche de medicaments.

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DIALOG(R) File 349:PCT Fulltext

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00613769 \*\*Image available\*\*

RETENTATE CHROMATOGRAPHY AND PROTEIN CHIP ARRAYS WITH APPLICATIONS IN BIOLOGY AND MEDICINE

CHROMATOGRAPHIE PAR RETENTAT ET ENSEMBLES DE DETECTION DE PROTEINES AYANT DES APPLICATIONS BIOLOGIQUES ET MEDICALES

Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, CIPHERGEN BIOSYSTEMS, INC. , Suite B, 470 San Antonio Road, Palo Alto, CA 94306 , US

Inventor(s):

HUTCHENS T William, HUTCHENS, T., William , 28 Carriage Court, Los Altos, CA 95618 , US

YIP Tai-Tung, YIP, Tai-Tung , 7515 Kingsbury Court, Cupertino, CA 95014 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9859361 A1 19981230

Application: WO 98US12907 19980619 (PCT/WO US9812907)

Priority Application: US 9754333 19970620; US 9767484 19971201

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FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

ML MR NE SN TD TG

Main International Patent Class: H01J-049/04;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 38957

#### English Abstract

This invention provides methods of retentate chromatography for resolving analytes in a sample. The methods involve adsorbing the analytes to a substrate under a plurality of different selectivity conditions, and detecting the analytes retained on the substrate by desorption spectrometry. The methods are useful in biology and medicine, including clinical diagnostics and drug discovery.

#### French Abstract

L'invention concerne des procedes de chromatographie par retentat permettant de detecter des analytes dans un echantillon. Ces procedes consistent a adsorber les analytes sur un substrat, dans une pluralite de conditions de selectivite differentes, et a detecter les analytes retenus sur ce substrat par spectrometrie de desorption. Ces procedes sont utiles en biologie et en medecine, notamment pour des diagnostics cliniques et la decouverte de medicaments.

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DIALOG(R)File 349:PCT Fulltext  
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00613768 \*\*Image available\*\*

RETENTATE CHROMATOGRAPHY AND PROTEIN CHIP ARRAYS WITH APPLICATIONS IN  
BIOLOGY AND MEDICINE

CHROMATOGRAPHIE PAR RETENTAT ET ENSEMBLES DE DETECTION DE PROTEINES AYANT  
DES APPLICATIONS EN BIOLOGIE ET EN MEDECINE

Patent Applicant/Assignee:

CIPHERGEN BIOSYSTEMS INC, CIPHERGEN BIOSYSTEMS, INC. , Suite B, 470 San  
Antonio Road, Palo Alto, CA 94306 , US

Inventor(s):

HUTCHENS T William, HUTCHENS, T., William , 28 Carriage Court, Los Altos,  
CA 95618 , US

YIP Tai-Tung, YIP, Tai-Tung , 7515 Kingsbury Court, Cupertino, CA 95014 ,  
US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9859360 A1 19981230

Application: WO 98US12843 19980619 (PCT/WO US9812843)

Priority Application: US 9754333 19970620; US 9767484 19971201

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
ML MR NE SN TD TG

Main International Patent Class: H01J-049/04;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 38891

#### English Abstract

This invention provides methods of retentate chromatography for resolving  
analytes in a sample. The methods involve adsorbing the analytes to a  
substrate under a plurality of different selectively conditions, and  
detecting the analytes retained on the substrate by desorption  
spectrometry. The methods are useful in biology and medicine, including  
clinical diagnostics and drug discovery.

#### French Abstract

Procede de chromatographie par retentat permettant de detecter des  
analytes dans un echantillon. Lesdits procedes consistent a adsorber les  
analytes sur un substrat dans une pluralite de conditions de selectivite  
differentes, et a detecter les analytes retenus sur le substrat par  
spectrometrie de desorption. Lesdits procedes sont utiles en biologie et  
en medecine, par exemple pour des diagnostics cliniques et la decouverte  
de medicaments.

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DIALOG(R)File 349:PCT Fulltext  
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00441062

METHODS FOR PRODUCING AND ANALYZING BIOPOLYMER LADDERS  
PROCEDES DE PRODUCTION ET D'ANALYSE D'ECHELLES DE BIOPOLYMERES

Patent Applicant/Assignee:

THE ROCKEFELLER UNIVERSITY

THE SCRIPPS RESEARCH INSTITUTE

CIPHERGEN BIOSYSTEMS INC

Inventor(s):

CHAIT Brian T

WANG Rong

HENT Stephen B

CLARK Steven M

Patent and Priority Information (Country, Number, Date):

Patent: WO 9636732 A1 19961121

Application: WO 96US6938 19960514 (PCT/WO US9606938)

Priority Application: US 95446208 19950519; US 95474997 19950607

Designated States: AU CA JP KR AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: C12Q-001/68;

International Patent Class: C12Q-001/34; C12Q-001/37; C12P-019/34;

G01N-033/00; C07H-019/00; C07H-021/02;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 6735

#### English Abstract

Methods of producing biopolymer ladders and their use to obtain structural information about the biopolymer. The ladders are produced by setting up catalytic cleavage and terminating reactions at the end of biopolymer molecules. The terminating reactions terminate cleavage of a percentage of the biopolymer molecules at each round of cleavage.

#### Japanese Abstract

L'invention se rapporte a des procedes de production d'echelles de biopolymeres et a leur utilisation permettant d'obtenir des informations structurelles sur le biopolymere. On produit ces echelles en etablissant un clivage catalytique et des reactions terminales a l'extremite des molecules des biopolymeres. Les reactions terminales terminent le clivage d'un pourcentage de molecules de biopolymeres a chaque serie de clivage.  
?e pa=large scale proteomics

Ref	Items	Index-term
E1	1	PA=LARGE SCALE BIOLOGY
E2	13	PA=LARGE SCALE BIOLOGY CORPORATION
E3	0	*PA=LARGE SCALE PROTEOMICS
E4	3	PA=LARGE SCALE PROTEOMICS CORP
E5	1	PA=LARGE SCALE PROTEOMICS CORPORATION
E6	2	PA=LARGE STORAGE CONFIGURATIONS INC
E7	2	PA=LARGE TERRY ARTHUR
E8	1	PA=LARGE TIMOTHY A
E9	5	PA=LARGE TIMOTHY ANDREW
E10	1	PA=LARGE WILLIAM THOMAS
E11	2	PA=LARGEAU
E12	1	PA=LARGEAU DENIS

Enter P or PAGE for more

?s e4-e5

3	PA=LARGE SCALE PROTEOMICS CORP
1	PA=LARGE SCALE PROTEOMICS CORPORATION
S3	4 E4-E5

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Estimated cost of output requested is: \$20.40

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3/5/1

DIALOG(R) File 349:PCT Fulltext

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00792521 \*\*Image available\*\*

GEL ELECTROPHORESIS IMAGE WARPING

DEFORMATION D'IMAGES PAR ELECTROPHORESE SUR GEL

Patent Applicant/Assignee:

LARGE SCALE PROTEOMICS CORP , 9620 Medical Center Drive, Rockville, MD

20850, US, US (Residence), US (Nationality)

Inventor(s):

TAYLOR John Jr, 401 Stonebrook Drive, Clayton, NC 27520, US,

Legal Representative:

LONGANECKER Stacey (et al) (agent), Roylance, Abrams, Berdo & Goodman,  
L.L.P., 1300 19th Street, N.W., Suite 600, Washington, DC 20036, US,  
Patent and Priority Information (Country, Number, Date):

Patent: WO 200126039 A1 20010412 (WO 0126039)

Application: WO 2000US27280 20001004 (PCT/WO US0027280)

Priority Application: US 99157830 19991005; US 2000643675 20000824

Designated States: AE AG AL AM AT AT (utility model) AU AZ BA BB BG BR BY  
BZ CA CH CN CR CU CZ CZ (utility model) DE DE (utility model) DK DK  
(utility model) DM DZ EE EE (utility model) ES FI FI (utility model) GB  
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KR (utility model) KZ LC LK  
LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK  
SK (utility model) SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G06K-009/00

International Patent Class: G06K-009/62; G06K-009/32; C12Q-001/68;  
C07H-021/02

Publication Language: English

Filing Language: English

English Abstract

A computer-implemented image processing method and apparatus for warping a plurality of gel electrophoresis images is provided. The method includes the step of assigning tiepoints in a reference image and in one or more object images (S3). The tiepoints in the object image are evaluated one-by-one by comparison to regions about a corresponding tiepoint in the reference image, and the location of the tiepoint in the object image is adjusted by slight movement to a location with respect to recognizable features in both the reference and object image thereby defining a tiepoint pair linking a location in the reference image with a location in the object image. Outlier tiepoint pairs may be rejected if that pair does not meet predetermined conditions (S3). Warping functions are generated and then globally optimized (S6). The plurality of images are tied together using the tiepoint pairs such that all of the images may be subsequently warped into registration to a single base image selected from the plurality of images (S10).

French Abstract

L'invention concerne un procede et un appareil de traitement d'images mises en oeuvre sur ordinateur destines a deformer une pluralite d'images par electrophorese sur gel. Ce procede comprend les etapes d'assignation de points de passage dans une image de reference et dans au moins une image objet (S3). Les points de passage dans l'image objet sont evalues un a un par comparaison a des regions situees autour d'un point de passage correspondant dans l'image de reference, et l'emplacement du point de passage dans l'image objet est ajuste par un leger mouvement a un emplacement en fonction de caracteristiques reconnaissables a la fois dans l'image objet et dans l'image de reference, ce qui permet ainsi de definir une paire de points de passage liant un emplacement dans l'image de reference avec un emplacement dans l'image objet. Des paires de points de passage aberrants peuvent etre rejetees si cette paire ne correspond pas aux conditions predeterminees (S3). Des fonctions de deformation sont generees et puis globalement optimisees (S6). La pluralite des images est rattachee ensemble au moyen des paires de points de passage, de telle maniere que toutes les images peuvent etre deformees posterieurement dans un enregistrement en fonction d'une image de base unique selectionnee a partir de plusieurs images (S10).

Legal Status (Type, Date, Text)

Publication 20010412 A1 With international search report.

3/5/2

DIALOG(R) File 349:PCT Fulltext

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00783332      \*\*Image available\*\*

**GEL ELECTROPHORESIS IMAGE COMBINING FOR IMPROVED DYNAMIC RANGE  
COMBINAISON D'IMAGES D'ELECTROPHORESE SUR GEL PERMETTANT D'OBTENIR UNE  
PLAGE DYNAMIQUE AMELIOREE**

Patent Applicant/Assignee:

**LARGE SCALE PROTEOMICS CORPORATION** , 9620 Medical Center Drive, Suite  
201, Rockville, MD 20850, US, US (Residence), US (Nationality)

Inventor(s):

TAYLOR John Jr, 401 Stonebrook Drive, Clayton, NC 27520, US,

Legal Representative:

IHNEN Jeffrey L (et al) (agent), Rothwell, Figg, Ernst & Manbeck, Suite  
701 East, 555 13th Street N.W., Columbia Square, Washington, DC 20004,  
US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200116884 A1 20010308 (WO 0116884)

Application: WO 2000US22885 20000821 (PCT/WO US0022885)

Priority Application: US 99387728 19990901

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DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ

LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG

SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G06T-001/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 4373

**English Abstract**

A computer-implemented image processing method and apparatus for combining a plurality of gel electrophoresis images. The method includes the steps of fitting the gel electrophoresis pixel intensity values for a subject pixel to a mathematical function, computing from the mathematical function a pixel intensity value according to a predetermined rule, and inserting the pixel intensity value into the composite image. The apparatus includes an image capturing device, and a computer having a memory and communicating with the image capturing device, the computer capable of receiving and storing into the memory a plurality of gel electrophoresis images from the image capturing device, the computer being further capable of fitting a plurality of intensity values of a pixel to a mathematical function over time and computing an optimal pixel intensity value for use in the composite image.

**French Abstract**

L'invention concerne un procede permettant de traiter une image mise en oeuvre par ordinateur et un appareil permettant de combiner plusieurs images d'electrophorese sur gel. Le procede consiste a adapter les valeurs d'intensite de pixel d'electrophorese sur gel pour un pixel sujet a une fonction mathematique, a calculer a partir de cette fonction mathematique une valeur d'intensite de pixel selon une regle determinee, et a inserer la valeur d'intensite de pixel dans l'image composite. L'appareil comprend un dispositif de saisie d'image, et un ordinateur pourvu d'une memoire communiquant avec ledit dispositif. L'ordinateur est en mesure de recevoir et de stocker dans sa memoire plusieurs images d'electrophorese sur gel a partir du dispositif de saisie d'image. Cet ordinateur est, en outre, capable d'adapter plusieurs valeurs d'intensite d'un pixel d'une fonction mathematique dans le temps, et de calculer une valeur d'intensite de pixel optimale pour une utilisation dans une image composite.

Legal Status (Type, Date, Text)

Publication 20010308 A1 With international search report.

DIALOG(R) File 349:PCT Fulltext  
(c) 2001 WIPO/MicroPat. All rts. reserv.

00783081 \*\*Image available\*\*

**DEVICES FOR USE IN MALDI MASS SPECTROMETRY**

**DISPOSITIFS DESTINES A ETRE UTILISES EN SPECTROMETRIE DE MASSE MALDI**

Patent Applicant/Assignee:

**LARGE SCALE PROTEOMICS CORP**, 9620 Medical Center Drive, Rockville, MD  
20850, US, US (Residence), US (Nationality)

Inventor(s):

ANDERSON N Leigh, 1759 Willard Street, N.W., Washington, DC 20009, US,  
GOODMAN Jack, P.O. Box 103, 1415 Crab House Road, Lusby, MD 20877, US,  
LENNON John, 18721 Capella Lane, Gaithersburg, MD 20657, US,

Legal Representative:

LONGANECKER Stacey (et al) (agent), Roylance, Abrams, Berdo & Goodman,  
L.L.P., 1300 19th Street, N.W., Suite 600, Washington, DC 20036, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200116574 A1 20010308 (WO 0116574)

Application: WO 2000US23167 20000824 (PCT/WO US0023167)

Priority Application: US 99151075 19990827

Designated States: AL AM AT AT (utility model) AU AZ BA BB BG BR BY CA CH  
CN CU CZ CZ (utility model) DE DE (utility model) DK DK (utility model)  
EE EE (utility model) ES FI FI (utility model) GB GD GE GH GM HR HU ID IL  
IN IS JP KE KG KP KR (utility model) KZ LC LK LR LS LT LU LV MD MG MK MN  
MW MX NO NZ PL PT RO RU SD SE SG SK SK (utility model) SL TJ TM TR TT UA  
UG UZ VN YU ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-001/10

International Patent Class: G01N-035/10; B01L-011/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 6361

**English Abstract**

An alignment plate (60) is provided with a plurality of holes (61) for guiding a pipette tip toward a sample plate (15) of a MALDI mass spectrometer. Each of the holes is provided with a conical upper contour (63) in order to guide the pipette tip toward a specific location on the sample plate (15). Two companion alignment plates are used in order to overlay two separate arrays of samples on the sample plate. For instance, a first of two alignment plates are used in order to overlay two separate arrays of samples on the sample plate. The first of two alignment plates is formed with a first array of holes so that a first array is deposited onto the sample plate. The second of the two alignment plates is formed with a second array of holes of samples is deposited on the sample plate at locations offset from the first array of samples already on the sample plate.

**French Abstract**

Une plaque d'alignement (60) est dotée d'une pluralité de trous (61) de guidage d'une pointe de pipette vers une plaque (15) d'échantillons d'un spectromètre de masse MALDI (desorption-ionisation par impact laser assistée par matrice). Chacun des trous présente un contour supérieur conique (63) afin de guider la pointe de la pipette vers un emplacement spécifique sur la plaque (15) d'échantillons. Deux plaques d'alignement complémentaires sont utilisées pour recouvrir deux réseaux séparés d'échantillons sur la plaque d'échantillons. Par exemple, une première des deux plaques d'alignement est utilisée pour recouvrir deux réseaux séparés d'échantillons sur la plaque d'échantillons. La première des deux plaques d'alignement est constituée d'un premier réseau de trous de manière qu'un premier réseau soit déposé sur la plaque d'échantillons. La seconde des deux plaques d'alignement présente un second réseau de trous d'échantillons déposé sur la plaque d'échantillons à des emplacements

decales du premier reseau d'echantillons se trouvant deja sur la plaque d'echantillons.

Legal Status (Type, Date, Text)

Publication 20010308 A1 With international search report.

3/5/4

DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00776993

**MICROARRAYS AND THEIR MANUFACTURE**

**BIOPUCES**

Patent Applicant/Assignee:

**LARGE SCALE PROTEOMICS CORP** , 9620 Medical Center Drive, Rockville, MD  
20850, US, US (Residence), US (Nationality)

Inventor(s):

ANDERSON Norman G, 10916 Wickshire Way, Rockville, MD 20852, US

ANDERSON N Leigh, 1759 Willard Street, N.W., Washington, DC 20009, US

BRAATZ James A, 4510 Yates Road, Beltsville, MD 20705, US

Legal Representative:

NAKAMURA Dean, Roylance, Abrams, Berdo & Goodman, LLP, Suite 600, 1300  
19th Street, N.W., Washington, DC 20036, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200109607 A1 20010208 (WO 0109607)

Application: WO 2000US20695 20000728 (PCT/WO US0020695)

Priority Application: US 99146653 19990730; US 2000482460 20000113

Designated States: AE AG AL AM AT AT (utility model) AU AZ BA BB BG BR BY  
BZ CA CH CN CR CU CZ CZ (utility model) DE DE (utility model) DK DK  
(utility model) DM DZ EE EE (utility model) ES FI FI (utility model) GB  
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KR (utility model) KZ LC LK  
LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK  
SK (utility model) SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-033/543

International Patent Class: G01N-033/551; G01N-033/552; G01N-033/566;

G01N-033/53; G01N-033/567; C12M-001/34; C12M-003/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23265

English Abstract

The instant invention relates to micro arrays containing bioreactive molecules, uses thereby, and methods for manufacture thereof. The arrays are constructed by sectioning bundles of tubules or rods, each containing unique reactants to produce large numbers of identical arrays.

French Abstract

L'invention concerne des puces contenant des molecules bioreactives, ainsi que les utilisations et les procedes de production de ces biopuces. On produit ces puces par sectionnement d'assemblages de tubules ou de tiges dont chacune contient un reactif distinct, de maniere a produire de grandes quantites de puces identiques.

Legal Status (Type, Date, Text)

Publication 20010208 A1 With international search report.

?e pa=phyllos

Ref	Items	Index-term
E1	2	PA=PHYLONIX
E2	2	PA=PHYLONIX PHARMACEUTICALS INC
E3	11	*PA=PHYLOS
E4	11	PA=PHYLOS INC

E5 86 PA=PHYSICAL  
E6 2 PA=PHYSICAL ELECTRONICS LABORATORY  
E7 33 PA=PHYSICAL OPTICS CORPORATION  
E8 15 PA=PHYSICAL SCIENCES INC  
E9 1 PA=PHYSICAL SYSTEMS INC  
E10 1 PA=PHYSICIAN  
E11 1 PA=PHYSICIAN VERIFICATION SERVICES INC  
E12 4 PA=PHYSICIANS

Enter P or PAGE for more

?s e3-e4

11 PA=PHYLOS  
11 PA=PHYLOS INC  
S4 11 E3-E4

?t 4/5/1-11

Estimated cost of output requested is: \$56.10  
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4/5/1

DIALOG(R)File 349:PCT Fulltext  
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00784060

METHODS FOR ENCODING AND SORTING IN VITRO TRANSLATED PROTEINS  
METHODES DE CODAGE ET DE TRI DE PROTEINES TRADUITES IN VITRO

Patent Applicant/Assignee:

PHYLOS INC, 128 Spring Street, Lexington, MA 02421, US, US (Residence),  
US (Nationality)

Inventor(s):

KUIMELIS Robert G, 21 Malbert Road, Brighton, MA 02135, US,

Legal Representative:

ELBING Karen L (agent), Clark & Elbing LLP, 176 Federal Street, Boston,  
MA 02110-02214, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200116352 A1 20010308 (WO 0116352)

Application: WO 2000US23414 20000825 (PCT/WO US0023414)

Priority Application: US 99151261 19990827

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ  
DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ  
LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG  
SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12P-021/06

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 8447

English Abstract

Described herein are methods and reagents for encoding and sorting in  
vitro translated proteins.

French Abstract

Methodes et reactifs destines a coder et a trier des proteines traduites  
in vitro.

Legal Status (Type, Date, Text)

Publication 20010308 A1 With international search report.

Publication 20010308 A1 With amended claims.

4/5/2



DIALOG(R)File 349:PCT Fulltext  
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00775234

**PEPTIDE ACCEPTOR LIGATION METHODS**  
**METHODES DE LIGATURE D'UN ACCEPTEUR DE PEPTIDE**

Patent Applicant/Assignee:

PHYLOS INC, 128 Spring Street, Lexington, MA 02421, US, US (Residence),  
US (Nationality)

Inventor(s):

KURZ Markus, 62 Kensington Street, West Newton, MA 02460, US  
LOHSE Peter, 50 Golden Ball Road, Weston, MA 02493, US  
WAGNER Richard, 1007 Lowell Road, Concord, MA 01742, US

Legal Representative:

ELBING Karen L, Clark & Elbing LLP, 176 Federal Street, Boston, MA  
02110-2214, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200107657 A1 20010201 (WO 0107657)

Application: WO 2000US19653 20000719 (PCT/WO US0019653)

Priority Application: US 99145834 19990727

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ

DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ

LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG

SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12Q-001/68

International Patent Class: C12P-021/06; C12P-019/34

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 10660

English Abstract

Described herein are methods and reagents for the ligation of a peptide  
acceptor to an RNA, as well as the RNA-peptide acceptor products.

French Abstract

L'invention concerne des methodes et des reactifs destines a fixer un  
accepteur de peptide a un ARN, ainsi que les produits accepteur de  
peptide - ARN ainsi obtenus.

Legal Status (Type, Date, Text)

Publication 20010201 A1 With international search report.

4/5/3

DIALOG(R)File 349:PCT Fulltext  
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00770881 \*\*Image available\*\*

**C-TERMINAL PROTEIN TAGGING**

**MARQUAGE DE PROTEINES C-TERMINALES**

Patent Applicant/Assignee:

PHYLOS INC, 128 Spring Street, Lexington, MA 02421, US, US (Residence),  
US (Nationality)

Inventor(s):

LOHSE Peter, 50 Golden Ball Road, Weston, MA 02493, US,  
MCPHERSON Michael, 142 Butler Avenue, Providence, RI 02906, US,  
KUIMELIS Robert G, 21 Malbert Road, Brighton, MA 02135, US,

Legal Representative:

ELBING Karen L (agent), Clark & Elbing LLP, 176 Federal Street, Boston,  
MA 02110-2214, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200104265 A2-A3 20010118 (WO 0104265)

Application: WO 2000US40347 20000711 (PCT/WO US0040347)

Priority Application: US 99143339 19990712  
Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ  
DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ  
LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG  
SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM  
Main International Patent Class: C12Q-001/68  
International Patent Class: C12N-015/00  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 4576

English Abstract

In general, the invention features proteins having covalently bonded  
C-terminal puromycin tags and methods for their production.

French Abstract

L'invention concerne, de maniere generale, des proteines ayant des  
etiquettes de puromycine C-terminale liees par covalence ainsi que leurs  
procedes de production.

Legal Status (Type, Date, Text)

Publication 20010118 A2 Without international search report and to be  
republished upon receipt of that report.

Search Rpt 20010405 Late publication of international search report

Republication 20010405 A3 With international search report.

4/5/4

DIALOG(R)File 349:PCT Fulltext

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00761080

**METHODS FOR PRODUCING 5'-NUCLEIC ACID-PROTEIN CONJUGATES**

**PROCEDES DE PRODUCTION DE CONJUGATS DE 5'-ACIDE NUCLEIQUE-PROTEINE**

Patent Applicant/Assignee:

PHYLOS INC, 128 Spring Street, Lexington, MA 02421, US, US (Residence),  
US (Nationality)

Inventor(s):

LOHSE Peter, 50 Golden Ball Road, Weston, MA 02493, US

WRIGHT Martin C, 812 Memorial Drive #1105, Cambridge, MA 02139, US

MCPHERSON Michael, 142 Butler Avenue, Providence, RI 02906, US

Legal Representative:

ELBING Karen L, Clark & Elbing, LLP, 176 Federal Street, Boston, MA  
02110-2214, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200072869 A1 20001207 (WO 0072869)

Application: WO 2000US15077 20000601 (PCT/WO US0015077)

Priority Application: US 99137032 19990601

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE  
DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC  
LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK  
SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: A61K-038/16

International Patent Class: A61K-038/03; C07K-014/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 5572

English Abstract

Disclosed herein is a method for generating a 5'-nucleic acid-protein conjugate, the method involving: (a) providing a nucleic acid which carries a reactive group at its 5' end; (b) providing a non-derivatized protein; and (c) contacting the nucleic acid and the protein under conditions which allow the reactive group to react with the N-terminus of the protein, thereby forming a 5'-nucleic acid-protein conjugate. Also disclosed herein are 5'-nucleic acid-protein conjugates and methods for their use.

French Abstract

L'invention a pour objet un procede pour generer un conjugat de 5'-acide nucleique-proteine. Ce procede consiste a (a) fournir un acide nucleique qui supporte un groupe reactif au niveau de son extremite 5'; (b) fournir une proteine non derivee, et (c) mettre en contact l'acide nucleique et la proteine dans des conditions qui permettent au groupe reactif de reagir avec la terminaison N de la proteine pour former ainsi un conjugat 5'-acide nucleique-proteine. L'invention traite egalement de conjugats de 5'-acide nucleique-proteine, et de procedes d'utilisation de ces derniers.

Legal Status (Type, Date, Text)

Publication 20001207 A1 With international search report.

Examination 20010412 Request for preliminary examination prior to end of 19th month from priority date

4/5/5

DIALOG(R) File 349:PCT Fulltext

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00721942

**PROTEIN SCAFFOLDS FOR ANTIBODY MIMICS AND OTHER BINDING PROTEINS**  
**ECHAFFAUDAGES DE PROTEINES POUR DES MIMES D'ANTICORPS ET AUTRES PROTEINES**  
**DE LIAISON**

Patent Applicant/Assignee:

PHYLOS INC, 128 Spring Street, Lexington, MA 02421, US, US (Residence),  
US (Nationality)

Inventor(s):

LIPOVSEK Dasa, 45 Sunset Road, Cambridge, MA 02138, US

Legal Representative:

ELBING Karen, Clark & Elbing LLP, 176 Federal Street, Boston, MA  
02110-2214, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200034784 A1 20000615 (WO 0034784)

Application: WO 99US29317 19991209 (PCT/WO US9929317)

Priority Application: US 98111737 19981210

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-033/536

International Patent Class: C07K-014/00; C12N-015/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 8786

English Abstract

Disclosed herein are proteins that include a fibronectin type III domain having at least one randomized loop. Also disclosed herein are nucleic

acids encoding such proteins and the use of such proteins in methods for evolving novel compound-binding species and their ligands.

#### French Abstract

L'invention concerne des proteines qui contiennent un domaine de fibronectine de type III comportant au moins une boucle aleatoire. L'invention concerne egalement des acides nucleiques codant ces proteines, ainsi que l'utilisation de ces proteines dans des methodes de developpement de nouvelles especes de liaison de composes, et leurs ligands.

#### Legal Status (Type, Date, Text)

Publication 20000615 A1 With international search report.  
Publication 20000615 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.  
Examination 20000908 Request for preliminary examination prior to end of 19th month from priority date  
Correction 20001116 Corrected version of Pamphlet: pages 1/12-12/12, drawings, replaced by new pages 1/12-12/12; due to late transmittal by the receiving Office

4/5/6

DIALOG(R) File 349:PCT Fulltext  
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00720106 \*\*Image available\*\*

#### DNA-PROTEIN FUSIONS AND USES THEREOF FUSIONS DE PROTEINE-ADN ET LEURS UTILISATIONS

Patent Applicant/Assignee:

PHYLOS INC, 128 Spring Street, Lexington, MA 02421, US, US (Residence),  
US (Nationality)

Inventor(s):

LOHSE Peter, 50 Golden Ball Road, Weston, MA 02493, US,  
KURZ Markus, 62 Kensington Street, West Newton, MA 02460, US,  
WAGNER Richard, 1007 Lowell Road, Concord, MA 01742, US,

Legal Representative:

ELBING Karen L (agent), Clark & Elbing LLP, 176 Federal Street, Boston,  
MA 02110-2214, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200032823 A1 20000608 (WO 0032823)  
Application: WO 99US28472 19991202 (PCT/WO US9928472)  
Priority Application: US 98110549 19981202

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12Q-001/68

International Patent Class: C12P-019/34; C07H-021/00; C07H-021/02;  
C07H-021/04

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 6983

#### English Abstract

The invention provides methods for covalently tagging proteins with their encoding DNA sequences as shown in the Figure. These DNA-protein fusions may be used in molecular evolution and recognition techniques.

#### French Abstract

L'invention concerne un procede permettant de marquer des proteines de

maniere covalente, ainsi que leurs sequences de codage d'ADN (voir figure). Ces fusions de proteines-ADN peuvent etre utilisees dans des techniques d'evolution et de reconnaissance moleculaire.

Legal Status (Type, Date, Text)  
Publication 20000608 A1 With international search report.  
Publication 20000608 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.  
Examination 20001130 Request for preliminary examination prior to end of 19th month from priority date  
Correction 20010329 Corrected version of Pamphlet: pages 1/20-20/20, drawings, replaced by new pages 1/20-20/20; due to late transmittal by the receiving Office  
Republication 20010329 A1 With international search report.

4/5/7

DIALOG(R) File 349:PCT-Fulltext  
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00705885

**SYNTHESIS OF CODON RANDOMIZED NUCLEIC ACIDS**

**SYNTHESE D'ACIDES NUCLEIQUES FONDEE SUR UNE GENERATION ALEATOIRE DE CODONS**

Patent Applicant/Assignee:

PHYLOS INC, PHYLOS, INC. , 128 Spring Street, Lexington, MA 02421 , US

Inventor(s):

LOHSE Peter, LOHSE, Peter , 50 Golden Ball Road, Weston, MA 02493 , US

KUIMELIS Robert G, KUIMELIS, Robert, G. , 21 Malbert Road, Brighton, MA 02135 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0018778 A1 20000406 (WO 200018778)

Application: WO 99US22436 19990928 (PCT/WO US9922436)

Priority Application: US 98102299 19980929

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE  
ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT  
UA UG UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ TZ UG ZW AM AZ BY KG KZ MD  
RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF  
CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C07H-001/00;

International Patent Class: C07H-001/02; C07C-255/11; C07C-255/49;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 13499

**English Abstract**

A method for generating a selected set of codons is disclosed; the method includes the steps of: (a) providing a first set of mononucleosides, mononucleotides, dinucleotides, or mixture thereof, where a subset A of the first set is protected with a protecting group A', and a subset B of the first set is protected with a protecting group B', where A' and B' are orthogonal protecting groups; (b) selectively removing the protecting group A' from subset A; (c) coupling the products of step (b) with a second set of mononucleosides, mononucleotides, dinucleotides, or a mixture thereof, where the second set is protected with protecting group A'; (d) optionally removing protecting group A' from the products of step (c); (e) optionally coupling the products of step (d) with a third set of mononucleosides, where the third set is protected with protecting group A'; (f) selectively removing the protecting group B' from subset B; (g) coupling the products of step (f) with a fourth set of mononucleosides, mononucleotides, dinucleotides, or a mixture thereof, where the fourth set is protected with protecting group A' or protecting group B'; (h) optionally selectively removing protecting group B' from the products of step (g); and (i) optionally coupling the products of step (h) with a fifth set of mononucleosides, to yield a selected set of codons.

#### French Abstract

L'invention se rapporte a un procede de generation d'un ensemble selectionne de codons. Ce procede consiste (a) a utiliser un premier ensemble constitue de mononucleosides, mononucleotides, dinucleotides ou d'un melange de ceux-ci, ledit premier ensemble comportant un sous-ensemble A protege par un groupe protecteur A' et un sous-ensemble B protege par un groupe protecteur B', A' et B' etant des groupes protecteurs orthogonaux; (b) a extraire selectivement le groupe protecteur A' du sous-ensemble A; (c) a coupler les produits de l'etape (b) a un second ensemble constitue de mononucleosides, mononucleotides, dinucleotides ou d'un melange de ceux-ci, ledit second ensemble etant protege par le groupe protecteur A'; (d) a extraire eventuellement le groupe protecteur A' des produits de l'etape (c); (e) a coupler eventuellement les produits de l'etape (d) a un troisieme ensemble de mononucleosides qui est protege par le groupe protecteur A'; (f) a extraire selectivement le groupe protecteur B' du sous-ensemble B; (g) a coupler les produits de l'etape (f) a un quatrieme ensemble constitue de mononucleosides, mononucleotides, dinucleotides ou d'un melange de ceux-ci, ledit quatrieme ensemble etant protege par le groupe protecteur A' ou le groupe protecteur B'; (h) a eventuellement extraire selectivement le groupe protecteur B' des produits de l'etape (g); et (i) a eventuellement coupler les produits de l'etape (h) a un cinquieme ensemble de mononucleosides, de maniere a produire un ensemble selectionne de codons.

4/5/8

DIALOG(R) File 349:PCT Fulltext

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00696924

**METHODS FOR PRODUCING NUCLEIC ACIDS LACKING 3'-UNTRANSLATED REGIONS AND OPTIMIZING CELLULAR RNA-PROTEIN FUSION FORMATION**  
**METHODES DE PRODUCTION D'ACIDES NUCLEIQUES PRIVES DE REGIONS NON TRADUITES 3' ET D'OPTIMISATION DE LA FORMATION D'UNE FUSION ARN CELLULAIRE-PROTEINE**

Patent Applicant/Assignee:

PHYLOS INC, PHYLOS, INC., 128 Spring Street, Lexington, MA 02421, US

Inventor(s):

HAMMOND Philip W, HAMMOND, Philip, W., 6 Hedgeway, Ayer, MA 01432, US  
LIPOVSEK Dasa, LIPOVSEK, Dasa, 45 Sunset Road, Cambridge, MA 02138, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0009737 A1 20000224 (WO 200009737)

Application: WO 99US18603 19990816 (PCT/WO US9918603)

Priority Application: US 9896818 19980817

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE  
ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT  
UA UG UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU  
TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG  
CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12P-019/34;

International Patent Class: C12N-015/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 8372

#### English Abstract

Described herein are methods for removing the 3'-untranslated regions from cDNA or mRNA molecules, as well as methods for the use of such products for RNA-protein fusion formation.

#### French Abstract

L'invention concerne des methodes d'elimination des regions non traduites 3' de molecules d'ADN complementaire ou d'ARN messenger, ainsi que des methodes d'utilisation de ces produits pour former une fusion

ARN-proteine.

Legal Status (Type, Date, Text)

Examination 20000602 Request for preliminary examination prior to end of  
19th month from priority date

4/5/9

DIALOG(R) File 349:PCT Fulltext

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00696710

IDENTIFICATION OF COMPOUND-PROTEIN INTERACTIONS USING LIBRARIES OF  
PROTEIN-NUCLEIC ACID FUSION MOLECULES

IDENTIFICATION D'INTERACTIONS COMPOSE-PROTEINE AU MOYEN DE BANQUES DE  
MOLECULES DE FUSION PROTEINE-ACIDE NUCLEIQUE

Patent Applicant/Assignee:

PHYLOS INC, PHYLOS, INC. , 128 Spring Street, Lexington, MA 02421 , US

Inventor(s):

LOHSE Peter, LOHSE, Peter , 28 Skahan Road, Belmont, MA 02178 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0009464 A1 20000224 (WO 200009464)

Application: WO 99US18600 19990816 (PCT/WO US9918600)

Priority Application: US 9896820 19980817

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE

ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT

LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT

UA UG UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU

TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG

CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C07B-061/00;

International Patent Class: C07K-001/04; C12Q-001/68; G01N-033/543;

G01N-033/551; G01N-033/552; G01N-033/544;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 4557

#### English Abstract

Disclosed herein is a method for detecting a compound-protein interaction, involving: (a) providing a compound library in which each member of the compound library is immobilized on a solid support; (b) contacting each member of the immobilized compound library in a single reaction chamber with each member of a protein-nucleic acid fusion library under conditions which allow the formation of compound-fusion complexes; (c) isolating the immobilized compound-fusion complexes; and (d) detecting a compound-fusion complex as an indication that the protein of the fusion interacts with the compound. In preferred embodiments, the protein is identified by reading the nucleic acid portion of the fusion, and the compound is identified by reading a detectable tag bound to either the compound or the solid support.

#### French Abstract

L'invention concerne une methode de detection d'une interaction compose-proteine consistant: (a) a prendre une banque de composes dans laquelle chaque element de la banque de composes est immobilise sur un support solide; (b) a mettre en contact chaque element de la banque de composes immobilises, dans une chambre de reaction unique, avec chaque element d'une banque de fusion proteine-acide nucleique dans des conditions favorisant la formation de complexes compose-produit de fusion; (c) a isoler les complexes compose-produit de fusion indiquant l'interaction de la proteine de la fusion avec le compose. Dans des modes de realisation preferes, on identifie la proteine en mesurant la partie d'acide nucleique de la fusion et on identifie le compose en mesurant un marqueur detectable lie au compose ou au support solide.

Legal Status (Type, Date, Text)

Examination 20000608 Request for preliminary examination prior to end of  
19th month from priority date  
Correction 20000928 Corrected version of Pamphlet: pages 1/4-4/4,  
drawings, replaced by new pages 1/4-4/4; due to late  
transmittal by the receiving Office

4/5/10  
DIALOG(R)File 349:PCT Fulltext  
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00687600

**METHODS FOR GENERATING HIGHLY DIVERSE LIBRARIES**  
**PROCEDES SERVANT A GENERER DES BANQUES EXTREMEMENT DIVERSIFIEES**

Patent Applicant/Assignee:

PHYLOS INC, PHYLOS, INC. , 128 Spring Street, Lexington, MA 02421 , US  
Inventor(s):

WAGNER Richard, WAGNER, Richard , 1007 Lowell Road, Concord, MA 01742 ,  
US

WRIGHT Martin C, WRIGHT, Martin, C. , 812 Memorial Drive & 1105,  
Cambridge, MA 02139 , US

KREIDER Brent, KREIDER, Brent , 4 Davis Road, Bedford, MA 01730 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0000632 A1 20000106 (WO 200000632)

Application: WO 99US14776 19990629 (PCT/WO US9914776)

Priority Application: US 9890970 19980629

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE

ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT

LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT

UA UG UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU

TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG

CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12P-021/06;

International Patent Class: C12P-019/34;-C12N-015/74;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 6121

**English Abstract**

Disclosed herein is a method for generating a nucleic acid library, the method involving: (a) providing a population of single- stranded nucleic acid templates, each of the templates including a coding sequence and an operably linked promoter sequence; (b) hybridizing to the population of single-stranded nucleic acid templates a mixture of substantially complementary single-stranded nucleic acid fragments, the fragments being shorter in length than the nucleic acid template; (c) contacting each of the hybridization products of step (b) with both a DNA polymerase which lacks strand displacement activity and a DNA ligase under conditions in which the fragments act as primers for the completion of a second nucleic acid strand which is substantially complementary to the nucleic acid template; and (d) contacting the products of step (c) with RNA polymerase to generate an RNA library, the library being transcribed from the second nucleic acid strand.

**French Abstract**

L'invention concerne un procede servant a generer une banque d'acides nucleiques et consistant a (a) mettre en application une population de gabarits d'acides nucleiques monocatenaires dont chacun comprend une sequence de codage et une sequence promoteur presentant une liaison operationnelle ; (b) hybrider a cette population de gabarits d'acides nucleiques monocatenaires un melange de fragments d'acides nucleiques monocatenaires pratiquement complementaires et dont la longueur est inferieure a celle du gabarit d'acide nucleique ; (c) mettre en contact chacun des produits d'hybridation de l'etape (b) avec a la fois une ADN polymerase a laquelle manque une activite de deplacement de brin et une ADN ligase dans des conditions dans lesquelles ces fragments jouent un



role d'amorces afin d'obtenir un deuxieme brin d'acide nucleique pratiquement complementaire du gabarit d'acide nucleique ; (d) mettre en contact les produits de l'etape (c) avec une ARN polymerase afin de generer une banque d'ARN transcrite depuis le deuxieme brin d'acide nucleique.

Legal Status (Type, Date, Text)

Examination 20000608 Request for preliminary examination prior to end of 19th month from priority date  
Correction 20001026 Corrected version of Pamphlet: pages 1/2-2/2, drawings, replaced by new pages 1/2-2/2; due to late transmittal by the receiving Office

4/5/11

DIALOG(R) File 349:PCT Fulltext

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00668271

ADDRESSABLE PROTEIN ARRAYS

SYSTEMES DE PROTEINES ADRESSABLES

Patent Applicant/Assignee:

PHYLOS INC, PHYLOS, INC. , 128 Spring Street, Lexington, MA 02421 , US

Inventor(s):

KUIMELIS Robert G, KUIMELIS, Robert, G. , 21 Malbert Road, Brighton, MA 02135 , US

WAGNER Richard, WAGNER, Richard , 1007 Lowell Road, Concord, MA 01742 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9951773 A1 19991014

Application: WO 99US7203 19990331 (PCT/WO US9907203)

Priority Application: US 9880686 19980403

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12Q-001/68;

International Patent Class: C12P-019/34; C07H-021/02; C07H-021/04;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 10059

English Abstract

Disclosed herein are arrays of nucleic acid-protein fusions which are immobilized to a solid surface through capture probes which include a non-nucleosidic spacer group and an oligonucleotide sequence to which the fusion (such as an RNA-protein fusion) is bound. Also disclosed herein are solid supports on which these arrays are immobilized as well as methods for their preparation and use (for example, for screening for protein-compound interactions such as protein-therapeutic compound interactions).

French Abstract

On decrit des systemes fusionnees d'acides nucleiques et de proteines, immobilises sur une surface solide a travers des sondes de capture incluant un groupe espaceur non nucleotidique, et une sequence oligonucleotidique a laquelle la fusion (fusion ARN-proteine, par exemple) est liee. On decrit egalement des supports solides sur lesquels ces systemes sont immobilises, ainsi que des methodes concernant leur preparation et utilisation (par exemple, pour un criblage destine a declencher des interactions proteine-compose, telles que des interactions d'une proteine et d'un compose therapeutique).

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00773597

**DERIVATIVE NUCLEIC ACIDS AND USES THEREOF**  
**ACIDES NUCLEIQUES MODIFIES ET UTILISATIONS ASSOCIEES**  
Patent Applicant/Assignee:

**PACKARD BIOSCIENCE COMPANY**, 800 Research Parkway, Meriden, CT  
06450-7169, US, US (Residence), US (Nationality), (For all designated  
states except: US

Patent Applicant/Inventor:

ENGLERT David F, 27 Rosedale Road, West Hartford, CT 06107, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

MYERS P Louis, Fish & Richardson P.C., 225 Franklin Street, Boston, MA  
02110-2804, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200106012 A1 20010125 (WO 0106012)

Application: WO 2000US19176 20000714 (PCT/WO US0019176)

Priority Application: US 99143804 19990714

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
QA BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM  
Main International Patent Class: C12Q-001/68  
International Patent Class: C12P-019/34  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 26385

English Abstract

The invention features a method for multiplexed analysis of a plurality of target nucleic acid sequences in a sample. The method provides a derivative nucleic acid for each target sequence analyzed and present in the sample.

French Abstract

L'invention concerne un procede d'analyse simultanee, dans un echantillon, de plusieurs sequences d'acides nucleiques cibles. Ce procede concerne un acide nucleique modifie, destine a chaque sequence cible analysee et presente dans l'echantillon.

Legal Status (Type, Date, Text)

Publication 20010125 A1 With international search report.

5/5/2

DIALOG(R)File 349:PCT Fulltext  
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00744622

**CONTINUOUS POROUS MATRIX ARRAYS**  
**RESEAUX MATRICIELS POREUX ET CONTINUS**

Patent Applicant/Assignee:

**PACKARD BIOSCIENCE COMPANY**, 800 Research Parkway, Meriden, CT 06450, US  
, US (Residence), US (Nationality), (For all designated states except:  
US

Patent Applicant/Inventor:

ENGLERT David F, 27 Rosedale Road, West Hartford, CT 06107, US, US  
(Residence), US (Nationality), (Designated only for: US)

NALLUR Girish N, 1 Marilyn Lane, Guilford, CT 06437, US, US (Residence),  
US (Nationality), (Designated only for: US)

HERBERT Alicia, 30 Beldenwood Road, Simsbury, CT 06437, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

MYERS P Louis, Fish & Richardson P.C., 225 Franklin Street, Boston, MA  
02110-2804, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200056934 A1 20000928 (WO 0056934)

Application: WO 2000US7796 20000324 (PCT/WO US0007796)

Priority Application: US 99125954 19990324

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE

DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC

LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK

SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12Q-001/68

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20198

English Abstract

Arrays of polymers on a continuous porous matrix array and methods of making and using same are disclosed.

French Abstract

Cette invention concerne des reseaux de polymeres disposes sur un reseau matriciel poreux et continu, ainsi que des procedes de fabrication et d'utilisation de ces reseaux.

Legal Status (Type, Date, Text)

Publication 20000928 A1 With international search report.  
Publication 20000928 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

5/5/3

DIALOG(R)File 349:PCT Fulltext

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00712279

IMPROVED PASSIVE NEUTRON COINCIDENCE AND MULTIPLICITY COUNTING  
COMPTAGE PERFECTIONNE DES MULTIPLICITES/COINCIDENCES DE NEUTRONS

Patent Applicant/Assignee:

PACKARD BIOSCIENCE COMPANY, PACKARD BIOSCIENCE COMPANY, 800 Research Parkway, Meriden, CT 06450, US

Inventor(s):

MCELROY Robert D Jr, MCELROY, Robert, D., Jr., 9 Blue Meadow Road, Middletown, CT 06457, US

VILLANI Marcel F, VILLANI, Marcel, F., 68 Victoria Boulevard, New Britain, CT 06052, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0025148 A2 20000504 (WO 200025148)

Application: WO 99US16956 19990727 (PCT/WO US9916956)

Priority Application: US 9894416 19980728

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE

ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT

LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT

UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD

RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF

CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: G01T-000/;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 3371

English Abstract

In a preferred embodiment, a method of enhancing the neutron coincidence assay of low fissile mass samples, by combining assay precision improvement algorithms with novel background and matrix correction algorithms, to provide lower levels of detection with higher confidence that the reported results are not subject to biases resulting from environmental or sample matrix effects.

French Abstract

Dans un mode de realisation prefere, l'invention concerne un procede d'accroissement de l'essai de coincidence de neutrons d'echantillons de masse a faible teneur en substance fissile, consistant a combiner des algorithmes de perfectionnement de precision d'essai avec des algorithmes nouveaux de correction de matrice et de fond naturel de rayonnement, afin d'obtenir des niveaux inferieurs de detection, en ayant la certitude que les resultats rapportes ne sont pas biaises par suite d'effets d'environnement ou de matrice d'echantillons.

Legal Status (Type, Date, Text)

Search Rpt 20000713 Late publication of international search report

5/5/4

DIALOG(R)File 349:PCT Fulltext  
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00706024

**METHOD FOR DETECTING ATP**

**METHODE DE DETECTION D'ADENOSINE-TRIPHOSPHATE (ATP)**

Patent Applicant/Assignee:

PACKARD BIOSCIENCE BV , PACKARD BIOSCIENCE B.V. , Ulgersmaweg 47,  
NL-9731 BK Groningen , NL

Inventor(s):

VAN LUNE Harry, VAN LUNE, Harry , Framaheerd 18, NL-9737 NL Groningen ,  
NL

TER WIEL Jan, TER WIEL, Jan , Molenweg 47, NL-9919 AG Loppersum , NL  
Patent and Priority Information (Country, Number, Date):

Patent: WO 0018953 A1 20000406 (WO 200018953)

Application: WO 99NL606 19990930 (PCT/WO NL9900606)

Priority Application: NL 1010224 19980930

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM  
TR TT TZ UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ TZ UG ZW AM AZ  
BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT  
SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12Q-001/66;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 5179

**English Abstract**

The invention relates to a method for detecting ATP in a sample, wherein the sample is contacted with a reaction mixture to effect a light signal, the reaction mixture comprising luciferin, luciferase and one or more water-soluble salts, the total salt concentration being at least 0.05 mole/liter, and wherein the light signal is measured. The invention further relates to a kit for use in this method.

**French Abstract**

L'invention concerne une methode de detection d'ATP dans un prelevement, la methode consistant a mettre en contact le prelevement avec un melange reactionnel pour produire un signal lumineux, le melange reactionnel comprenant de la luciferine, de la luciferase et un ou plusieurs sels solubles dans l'eau et la concentration totale en sel s'elevant a au moins 0,05 moles/litre; puis a mesurer le signal lumineux. L'invention concerne en outre une trousse destinee a s'utiliser avec cette methode.

Legal Status (Type, Date, Text)

Examination 20000615 Request for preliminary examination prior to end of  
19th month from priority date

5/5/5

DIALOG(R)File 349:PCT Fulltext  
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00696925

**ROLLING CIRCLE-BASED ANALYSIS OF POLYNUCLEOTIDE SEQUENCE**

**ANALYSE PAR CERCLES ROULANTS D'UNE SEQUENCE POLYNUCLEOTIDIQUE**

Patent Applicant/Assignee:

PACKARD BIOSCIENCE COMPANY , PACKARD BIOSCIENCE COMPANY , 800 Research  
Parkway, Meriden, CT 06450 , US

Inventor(s):

WOODWARD Karen L, WOODWARD, Karen, L. , 3 Woodward Road, Columbia, CT  
06237 , US

NALLUR Girish N, NALLUR, Girish, N. , 599D Prospect Street, New Haven, CT  
06511 , US

TAYLOR Seth, TAYLOR, Seth , Apartment 2-9B, 100 Memorial Drive,  
Cambridge, MA 02142 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0009738 A1 20000224 (WO 200009738)  
Application: WO 99US18808 19990817 (PCT/WO US9918808)

Priority Application: US 9896830 19980817; US 98102535 19980930; US  
98106910 19981103; US 98106885 19981103

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM  
TR TT UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG  
KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF  
BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12P-019/34;

International Patent Class: C12N-015/00; C07H-021/00; C07H-021/02;  
C07H-021/04;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 33384

English Abstract

Disclosed are methods of detecting a polynucleotide sequence.

French Abstract

La presente invention concerne des procedes de detection d'une sequence  
polynucleotidique.

Legal Status (Type, Date, Text)

Correction 20000518 Corrected version of Pamphlet: pages 1/12-12/12,  
drawings, replaced by new pages 1/9-9/9; due to late  
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Examination 20000615 Request for preliminary examination prior to end of  
19th month from priority date

5/5/6

DIALOG(R)File 349:PCT Fulltext

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00675230

**DIGITAL PULSE DE-RANDOMIZATION FOR RADIATION SPECTROSCOPY**  
**DERANDOMISATION NUMERIQUE D'IMPULSIONS POUR SPECTROSCOPIE DE RAYONNEMENTS**

Patent Applicant/Assignee:

**PACKARD BIOSCIENCE COMPANY** , PACKARD BIOSCIENCE COMPANY , 800 Research  
Parkway, Meriden, CT 06450 , US

Inventor(s):

JORDANOV Valentin T, JORDANOV, Valentin T. , 12 Cutts Road, Durham, NH  
03824 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9958999 A1 19991118

Application: WO 99US10010 19990507 (PCT/WO US9910010)

Priority Application: US 9884891 19980509

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU  
LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA  
UG US UZ VN YU ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ  
TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI  
CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: G01T-001/17;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 4525

English Abstract

In a preferred embodiment, a method of digitally de-randomizing pulses in a radiation spectroscopy system, the method including the steps of: receiving an input signal representative of a radiation detector output; analyzing the input signal to derive separate event samples and background samples; storing the event samples and the background samples; and reading stored event samples and background samples and adjusting spacing in time between adjacent event samples such that the event samples are spaced apart a time interval at least equal to pulse processing time of elements receiving an output of spaced apart event samples and the background samples.

French Abstract

Selon un mode de realisation prefere, l'invention concerne un procede de derandomisation numerique d'impulsions dans un systeme de spectroscopie. Ledit procede consiste a: recevoir un signal d'entree representatif d'une sortie de detecteur de rayonnements; a analyser le signal d'entree pour en deduire des echantillons d'evenements et des echantillons de fond separees; a enregistrer les echantillons d'evenements et les echantillons de fond; et a lire les echantillons d'evenements et les echantillons de fond enregistres et a ajuster l'espacement dans le temps entre des echantillons d'evenements adjacents de maniere que les echantillons d'evenements soient separees d'un intervalle de temps au moins egal aux temps de traitement d'impulsions des elements recevant une sortie d'echantillons d'evenements separees et les echantillons de fond.

5/5/7

DIALOG(R) File 349:PCT Fulltext

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00669498

ANALYSIS OF POLYNUCLEOTIDE SEQUENCE

ANALYSE DE SEQUENCE DE POLYNUCLEOTIDES

Patent Applicant/Assignee:

PACKARD BIOSCIENCE COMPANY , PACKARD BIOSCIENCE COMPANY , 800 Research Parkway, Meriden, CT 06450 , US

Inventor(s):

TAYLOR Seth, TAYLOR, Seth , Apartment 2-9B, 100 Memorial Drive, Cambridge, MA 02142 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9953102 A1 19991021

Application: WO 99US8407 19990416 (PCT/WO US9908407)

Priority Application: US 9882063 19980416; US 9884085 19980507

Designated States: AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: C12Q-001/68;

International Patent Class: C12P-019/34; C07H-021/02; C07H-021/04;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 14335

English Abstract

Disclosed are methods for detecting nucleic acids using rolling circle-based amplification and arrays of capture probes.

French Abstract

La presente invention concerne des procedes permettant de detecter des acides nucleiques mettant en oeuvre une amplification utilisant le principe des cercles roulants et des matrices de sondes de capture.

5/5/8

DIALOG(R) File 349:PCT Fulltext

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00653542

**GEL PAD ARRAYS AND METHODS AND SYSTEMS FOR MAKING THEM**  
**ENSEMBLE DE MATRICES DE GEL ET SYSTEME DE FABRICATION DE TELS ENSEMBLES**

Patent Applicant/Assignee:

**PACKARD BIOSCIENCE COMPANY** , **PACKARD BIOSCIENCE COMPANY** , 800 Research  
Parkway, Meriden, CT 06450 , US

Inventor(s):

**TAYLOR Seth**, **TAYLOR, Seth** , Apartment 2-9B, 100 Memorial Drive,  
Cambridge, MA 02142 , US

**CROKER Kevin**, **CROKER, Kevin** , 1271 Lilac Court, Cheshire, CT 06410 , US

**WEBER Shane Crawford**, **WEBER, Shane**, Crawford , One Evergreen Drive,  
Woodbridge, CT 06525 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9936576 A1 19990722

Application: WO 99US1170 19990120 (PCT/WO US9901170)

Priority Application: US 9871980 19980120; US 9875698 19980121; US  
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FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV

MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG

US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT

BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA

GN GW ML MR NE SN TD TG

Main International Patent Class: C12Q-001/68;

International Patent Class: C07H-021/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 13125

English Abstract

Gel pads and gel pad arrays, and methods for making and using them, are  
disclosed. The gel pads preferably comprise an intelligent gel.

French Abstract

La presente invention concerne des matrices de gel et des ensembles de  
matrices de gel, les procedes de fabrication et leur utilisation. Les  
matrices de gel contiennent, de preference, un gel intelligent.

5/5/9

DIALOG(R) File 349:PCT Fulltext

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00647902 \*\*Image available\*\*

**MICROTITER PLATE WITH COATING**

**PLAQUE DE MICROTITRAGE POURVUE D'UN REVETEMENT**

Patent Applicant/Assignee:

**PACKARD BIOSCIENCE BV** , **PACKARD BIOSCIENCE B.V.** , Ulgersmaweg 47,  
NL-9731 BK Groningen , NL

Inventor(s):

**THOMSON James**, **THOMSON, James** , 110 Steeplechase Drive, Newington, CT  
06111 , US

**TER WIEL Jan**, **TER WIEL, Jan** , Molenweg 47, NL-9919 AG Loppersum , NL

Patent and Priority Information (Country, Number, Date):

Patent: WO 9930823 A1 19990624

Application: WO 98NL711 19981211 (PCT/WO NL9800711)

Priority Application: NL 107781 19971212

Designated States: JP US AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT  
SE

Main International Patent Class: B01L-003/00;

International Patent Class: G01N-035/02; B05D-007/24; B05D-003/14;

C08F-002/00; C08J-007/04;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description



Claims  
Fulltext Word Count: 3614

English Abstract

The invention relates to a plastic microtiter plate provided with a coating which comprises a para-xylylene polymer, and to a method for applying such a coating to a plastic microtiter plate. The invention also relates to the use of such a microtiter plate in carrying out chemical, biochemical, biological, clinical or pharmaceutical analyses.

French Abstract

L'invention concerne une plaque de microtitrage en plastique pourvue d'un revêtement composé d'un polymère de para-xylylene, ainsi qu'un procédé servant à appliquer ce revêtement à une plaque de microtitrage en plastique. Elle concerne également l'utilisation de cette plaque de microtitrage afin d'exécuter des analyses chimiques, biochimiques, biologiques, cliniques ou pharmaceutiques.

5/5/10

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00645386

**METHODS OF USING PROBES FOR ANALYZING POLYNUCLEOTIDE SEQUENCE**  
**PROCEDES D'UTILISATION DE SONDES PERMETTANT D'ANALYSER UNE SEQUENCE DE POLYNUCLEOTIDE**

Patent Applicant/Assignee:

**PACKARD BIOSCIENCE COMPANY**, PACKARD BIOSCIENCE COMPANY, 800 Research Parkway, Meriden, CT 06450, US

Inventor(s):

TAYLOR Seth, TAYLOR, Seth, Apartment 2-9B, 100 Memorial Drive, Cambridge, MA 02142, US

VAN CAUTER Staf, VAN CAUTER, Staf, 145 Tuttle Point Road, Guilford, CT 06437, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9928494 A1 19990610

Application: WO 98US25664 19981203 (PCT/WO US9825664)

Priority Application: US 9767517 19971204; US 9769642 19971215; US 9871443 19980114

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12Q-001/06;

International Patent Class: C12P-019/34; C07H-021/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 11450

English Abstract

A method of detecting the presence of, or identifying, a genetic event comprising: providing a target nucleic acid having a genetic event; providing a plurality of nucleotide probes wherein each probe: (a) is complementary to a region immediately 5' to the site of the genetic event and complementary to a region immediately 3' to the site of the genetic event, (b) has an identification region, e.g., a 5' terminal region or a 3' terminal region, which identification region is not repeated in another probe in the plurality, i.e., it is unique in the plurality, and is complementary to the target sequence, and (c) wherein the nucleotide at the position corresponding to the site of the first genetic event can be chosen from A, T, G or C, wherein the plurality of nucleotide probes includes a first probe having a first base at the position corresponding to the site of the genetic event and a second probe having a second

(i.e., different from the first base) base at the position corresponding to the site of the genetic event; contacting the target with the plurality of probes under conditions wherein only probes having complementarity with the target nucleic acid will hybridize; thereby analysing the target nucleic acid.

#### French Abstract

L'invention concerne un procede permettant de detecter la presence d'un evenement genetique ou de l'identifier. Ce procede consiste a fournir un acide nucleique cible presentant un evenement genetique; a fournir une pluralite de sondes de nucleotide dans lequel a) chaque sonde est complementaire d'une region 5' immediate du site de l'evenement genetique et complementaire d'une region 3' immediate de l'evenement genetique, b) chaque sonde possede une region d'identification, par exemple une region terminale 5'-terminale ou une region 3'-terminale qui n'est pas repetee dans une autre sonde de la pluralite, c'est-a-dire, qu'elle est unique dans la pluralite et complementaire de la sequence cible, et c) le nucleotide a la position correspondant au site du premier evenement genetique peut etre choisi dans A, T, G, ou C, la pluralite de sondes de nucleotide comprenant une premiere sonde dotee d'une premiere base en position correspondant au site de l'evenement genetique et une seconde sonde dotee d'une seconde base (differente de la premiere) en position correspondant au site de l'evenement genetique; a mettre en contact la cible avec la pluralite de sondes dans des conditions ou seules les sondes complementaires de l'acide nucleique cible sont hybridisees; a analyser l'acide nucleique.

?e pa=cellomics

Ref	Items	Index-term
E1	1	PA=CELLOMEDA
E2	1	PA=CELLOMEDA OY
E3	16	*PA=CELLOMICS
E4	16	PA=CELLOMICS INC
E5	1	PA=CELLOMICSTM
E6	1	PA=CELLOMICSTM INC
E7	7	PA=CELLPACK
E8	4	PA=CELLPACK AG
E9	3	PA=CELLPACK GMBH
E10	1	PA=CELLPATH
E11	1	PA=CELLPATH INC
E12	1	PA=CELLPEP

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16	PA=CELLOMICS
16	PA=CELLOMICS INC
1	PA=CELLOMICSTM
1	PA=CELLOMICSTM INC
17	E3-E6

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00778739

#### A SYSTEM FOR CELL-BASED SCREENING SYSTEME CRIBLAGE DE CELLULES

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

OLSON Keith, 622 College Street, Pittsburgh, PA 15232, US, US (Residence)  
, US (Nationality), (Designated only for: US )

LAPETS Oleg, 1937 Shady Oak Circle, Allison Park, PA 15101, US, US

(Residence), US (Nationality), (Designated only for: US )  
BRIGHT Gary, 4649 Hidden Pond, Allison Park, PA 15101, US, US (Residence)  
, US (Nationality), (Designated only for: US )  
SHOPOFF Randall O, 9 New London Lane, Oakmont, PA 15139, US, US  
(Residence), US (Nationality), (Designated only for: US )

Legal Representative:

HARPER David S, McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker  
Drive, Suite 3200, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200111341 A2 20010215 (WO 0111341)

Application: WO 2000US21426 20000804 (PCT/WO US0021426)

Priority Application: US 99147254 19990805; US 99398965 19990917; US  
2000174356 20000104; US 2000176715 20000118

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

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Main International Patent Class: G01N-015/14

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Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 32391

English Abstract

The present invention provides systems, methods, screens, reagents and  
kits for optical system analysis of cells to rapidly determine the  
distribution, environment, or activity of fluorescently labeled reporter  
molecules in cells for the purpose of screening large numbers of  
compounds for those that specifically affect the mitotic index of cells.

French Abstract

L'invention concerne des systemes, des techniques, des cribles, des  
reactifs, et des kits destines a l'analyse de cellules d'un systeme  
optique, afin de determiner rapidement la repartition, l'environnement,  
ou l'activite de molecules reporteurs marquees par fluorescence dans des  
cellules, et de cribler un grand nombre de composes qui affectent de  
maniere specifique l'indice mitotique desdites cellules.

Legal Status (Type, Date, Text)

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6/5/2

DIALOG(R) File 349:PCT Fulltext

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00777839 \*\*Image available\*\*

**OPTICAL SYSTEM ANALYSIS OF CELLS**

**ANALYSE DE CELLULES PAR SYSTEME OPTIQUE**

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

GHOSH Richik, 2134 Mountain Way Lane, Monroeville, PA 15146, US, IN

(Residence), IN (Nationality), (Designated only for: US )

DEBIASIO Robin L, 53 Locust Lane, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (Designated only for: US )

JANARDHAN Prem, 635 William Pitt Way, Pittsburgh, PA 15238, US, IN

(Residence), IN (Nationality), (Designated only for: US )

Legal Representative:

HARPER David S, McDonnell, Boehnen, Hulbert & Berghoff, Suite 3200, 300  
South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

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Priority Application: US 99147443 19990805; US 99398965 19990917; US 2000176589 20000118; US 2000205696 20000519

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-015/14

International Patent Class: G01N-033/53

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 36431

#### English Abstract

The present invention provides systems, methods, screens, reagents and kits for optical system analysis of cells to rapidly determine the distribution, environment, or activity of fluorescently labeled reporter molecules in cells for the purpose of screening large numbers of compounds for those that specifically affect neurite outgrowth.

#### French Abstract

La presente invention concerne des systemes, des filtres, des reactifs et des kits pour l'analyse de cellules par systeme optique qui permettent de determiner rapidement la distribution, l'environnement ou l'activite de molecules reporteurs marquees par fluorescence dans des cellules en vue de cribler de grands nombres de composes pour trouver ceux qui affectent specifiquement la formation des neurites.

#### Legal Status (Type, Date, Text)

Publication 20010215 A1 With international search report.

Publication 20010215 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

6/5/3

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00775306

#### METHOD AND SYSTEM FOR DYNAMIC STORAGE AND VALIDATION OF RESEARCH DATA PROCEDE ET SYSTEME D'ENREGISTREMENT DYNAMIQUE ET DE VALIDATION DE DONNEES DE RECHERCHE

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15328, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

QU Long, 2616 Patrice Court, Murrysville, PA 15668, US, US (Residence), CN (Nationality), (Designated only for: US )

WANG Jian, 401 Shady Avenue, Pittsburgh, PA 15206, US, US (Residence), CN (Nationality), (Designated only for: US )

HARRINGTON Christopher C, 4 Edgewood Road, Pittsburgh, PA 15215, US, US (Residence), US (Nationality), (Designated only for: US )

TAYLOR D Lansing, 910 Notre Dame Place, Pittsburgh, PA 15215, US, US (Residence), US (Nationality), (Designated only for: US )

RAAB CARSON Mandy M, 410 Lenox Avenue, Pittsburgh, PA 15221, US, US (Residence), US (Nationality), (Designated only for: US )

Legal Representative:

LESAVICH Stephen, McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

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Application: WO 2000US20287 20000726 (PCT/WO US0020287)  
Priority Application: US 99145770 19990727

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DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G06F-017/00

Publication Language: English

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Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 6699

English Abstract

A method and system for sharing validated experimental data (e.g., cell experimental data). Raw experimental data is accepted and divided into one or more raw data components using a modifiable data object model. The modifiable data object model includes a small hierarchical structure that does not waste storage space and can be expanded by users. The modifiable data object model can be modified by a user to add raw experimental data for virtually any experiment conducted by a user. The raw experimental data is validated and if desired made available to other researchers via a publicly accessible server on a computer network (e.g., the Internet), thereby allowing the validated experimental data to be shared by researchers via the computer network. The publicly accessible server is associated with a knowledge repository for accumulating a body of knowledge for researchers in a particular field (e.g., cell biology). The raw experimental data can also be automatically validated with a pre-determined validation process to create validated experimental data. The validated experimental data is made available immediately after validation on a publicly accessible server on a computer network. The present invention may also be used to further facilitate a user's understanding of biological functions, such as cell functions, to design experiments more intelligently and to analyze experimental results more thoroughly by making raw and validated experimental data immediately available via a computer network after input. Specifically, the present invention may help drug discovery scientists select better targets for pharmaceutical intervention in the hope of curing diseases. The method and system may also help facilitate the abstraction of knowledge from information for biological experimental data and provide new bioinformatic techniques.

French Abstract

La presente invention concerne un procede et un systeme de partage de donnees experimentales validees, telles que des donnees experimentales de biologie cellulaire. Ces donnees experimentales brutes sont prises en compte et divisees en une ou plusieurs composantes de donnees brutes par utilisation d'un modele modifiable d'objet de donnees. Ce modele modifiable d'objet de donnees comporte une petite structure hierarchique qui ne gaspille pas l'espace en memoire et qui peut etre developpee par les utilisateurs. Le modele modifiable d'objet de donnees peut etre modifie par un utilisateur de facon a ajouter des donnees experimentales brutes pour virtuellement toute experience menee par un utilisateur. La donnee experimentale brute est validee, et si on le souhaite, elle est rendue disponible pour d'autres chercheurs via un serveur accessible de facon publique sur un reseau d'ordinateurs tel que l'Internet, ce qui permet le partage entre chercheurs des donnees experimentales validees via le reseau d'ordinateurs. Ce serveur accessible de facon publique est associe a un fond d'archivage de connaissances destine aux chercheurs d'un domaine particulier tel que la biologie cellulaire. Les donnees experimentales brutes peuvent egalement etre automatiquement validees au moyen d'un processus de validation prealablement defini, de facon a creer

des donnees experimentales validees. Les donnees experimentales validees sont rendues disponibles immediatement apres validation sur un serveur accessible de facon publique sur un reseau d'ordinateurs. La presente invention permet egalement une meilleure comprehension par les utilisateurs de fonctions biologiques telles que les fonctions cellulaires, de facon a concevoir de facon plus intelligente les experiences, et pour analyser les resultats experimentaux plus completement en rendant les donnees experimentales brutes et validees immediatement disponible via un reseau d'ordinateurs apres la saisie. De facon plus specifique, la presente invention peut aider les chercheurs en pharmacie a mieux selectionner les cibles des interventions pharmaceutiques dans avec l'espoir de guerir des maladies. Le procede et le systeme peuvent egalement favoriser l'extraction de donnees biologiques experimentales a partir des connaissances et procurer de nouvelles techniques de bioinformatique.

Legal Status (Type, Date, Text)

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6/5/4

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00775277

**MINIATURIZED CELL ARRAY METHODS AND APPARATUS FOR CELL-BASED SCREENING**  
**METHODES ET APPAREIL DE CRIBLAGE SUR LA BASE DE CELLULES AVEC UN RESEAU**  
**MINIATURISE DE CELLULES**

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

KAPUR Ravi, 2942 E. Bardoneer Road, Gibsonsia, PA 15044, US, IN

(Residence), IN (Nationality), (Designated only for: US )

GIULIANO Kenneth, 351 Hawthorne Road, Pittsburgh, PA 15209, US, US

(Residence), US (Nationality), (Designated only for: US )

Legal Representative:

HARPER David S, McDonnell, Boehnen, Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200107889 A2 20010201 (WO 0107889)

Application: WO 2000US20003 20000721 (PCT/WO US0020003)

Priority Application: US 99145757 19990727; US 99401212 19990922; US

2000513783 20000225; US 2000540862 20000331

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DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ

TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

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Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 43445

English Abstract

The present invention describes methods and cassettes for cell-based toxin detection and organ localization. The cassettes includes an array containing cells and a matrix of openings or depressions, wherein each region of the substrate enclosed by the opening or depression in the matrix forms a domain individually addressable by microfluidic channels in the device.

French Abstract

L'invention concerne des methodes et des cassettes destinees a la localisation d'organes et a la detection de toxine sur la base de cellules. Ces cassettes comprennent un reseau contenant des cellules et une matrice constituee d'ouvertures ou de creux. Chaque region du substrat renfermee par une ouverture ou un creux de la matrice forme un domaine adressable individuellement par des canaux microfluidiques situes dans le dispositif.

Legal Status (Type, Date, Text)

Publication 20010201 A2 Without international search report and to be republished upon receipt of that report.

6/5/5

DIALOG(R) File 349:PCT Fulltext

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00774376 \*\*Image available\*\*

MINIATURIZED CELL ARRAY METHODS AND APPARATUS FOR CELL-BASED SCREENING  
PROCEDES ET APPAREIL DE JEU ORDONNE MINIATURISE DE CELLULES DESTINES AU  
CRIBLAGE CELLULAIRE

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US  
(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

KAPUR Ravi, 2942 E. Bardoneer Road, Gibsonia, PA 15044, US, US  
(Residence), US (Nationality), (Designated only for: US )

ADAMS Terri, 100 Eastern Avenue, Pittsburgh, PA 15215, US, US (Residence)  
, US (Nationality), (Designated only for: US )

Legal Representative:

HARPER David S, McDonnell, Boehnen, Hulbert & Berghoff, 300 South Wacker  
Drive, Suite 3200, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200107891 A2 20010201 (WO 0107891)

Application: WO 2000US20407 20000727 (PCT/WO US0020407)

Priority Application: US 99145884 19990727; US 99401212 19990922; US  
2000540862 20000331

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK

DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ

TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

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Main International Patent Class: G01N-015/00

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Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 37502

English Abstract

The present invention describes array for cell screening, and methods for making them, comprising a substrate with three dimensional hydrated polymer constructs in the reaction wells, wherein the hydrated polymer suspension comprises cell adhesion promoters and cells to be analyzed, and wherein the substrate further comprises inter-well spaces comprising one or more cell adhesion inhibitors.

French Abstract

La presente invention concerne un jeu ordonne de microechantillons destine au criblage cellulaire et son procede de fabrication, lequel jeu de microechantillons comprend un substrat muni de puits de reaction dans lesquels se trouvent des constructions de polymere hydrate en trois dimensions, la suspension de polymere hydrate comprenant des promoteurs d'adhesion cellulaire et des cellules a analyser, et le substrat

comprenant en outre au moins un inhibiteur d'adhesion cellulaire dans les espaces separant les puits.

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6/5/6

DIALOG(R) File 349:PCT Fulltext

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00766801

A SYSTEM FOR CELL-BASED SCREENING

SYSTEME D'EXAMEN SYSTEMATIQUE DE CELLULES

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

RUBIN Richard A, 5866 Bartlett Street, Pittsburgh, PA 15217, US, US

(Residence), US (Nationality), (Designated only for: US )

GOUGH Albert H, 2014 Louise Drive, Glenshaw, PA 15116, US, US (Residence)

, US (Nationality), (Designated only for: US )

GHOSH Richik N, 2134 Mountain Way Lane, Monroeville, PA 15146, US, US

(Residence), IN (Nationality), (Designated only for: US )

GIULIANO Kenneth A, 351 Hawthorne Road, Pittsburgh, PA 15209, US, US

(Residence), US (Nationality), (Designated only for: US )

DUNLAY R Terry, 433 Lynn Ann Drive, New Kensington, PA 15068, US, US

(Residence), US (Nationality), (Designated only for: US )

Legal Representative:

HARPER David S, McDonnell, Boehnen, Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200079241 A2 20001228 (WO 0079241)

Application: WO 2000US40260 20000621 (PCT/WO US0040260)

Priority Application: US 99140143 19990621; US 99352171 19990712; US 99148360 19990811; US 99170313 19991213

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DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ

TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

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Main International Patent Class: G01N-015/14

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Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 32781

English Abstract

The present invention provides methods, computer readable storage medium, and kits for identifying compounds that induce or inhibit macromolecule trafficking through the endosomal system, comprising treating cells that possess a luminescently-tagged macromolecule with a test compound, obtaining luminescent signals from the cells, converting the luminescent signals into digital data, and utilizing the digital data to determine whether the test compound has induced or inhibited macromolecule trafficking through the endosomal system.

French Abstract

La presente invention porte sur des procedes, sur support d'enregistrement lisible par un ordinateur et des kits permettant d'identifier des composees qui induisent ou inhibent le trafic de macromolecules dans le systeme endosomal. Ces procedes consistent a traiter avec un compose de test des cellules qui possedent une



macromolecule marquee par luminescence, generer des signaux luminescents a partir de ces cellules, convertir les signaux en donnees numeriques et utiliser les donnees numeriques pour determiner si le compose de test a induit ou inhibe le trafic de macromolecules dans le systeme endosomal.

Legal Status (Type, Date, Text)

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6/5/7

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00759633

**METHOD AND SYSTEM FOR GENERAL PURPOSE ANALYSIS OF EXPERIMENTAL DATA  
PROCEDE ET SYSTEME POUR ANALYSER DE MANIERE POLYVALENTE DES DONNEES  
EXPERIMENTALES**

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15328, US, US  
(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

GOUGH Albert H, 2014 Louise Drive, Glenshaw, PA 15116, US, US (Residence)  
, US (Nationality), (Designated only for: US )

LAPETS Oleg P, 1937 Shady Oak Circle, Allison Park, PA 15101, US, US  
(Residence), RU (Nationality), (Designated only for: US )

BRIGHT Gary, 586 Sturbridge Drive, Highland Heights, OH 44143, US, US  
(Residence), US (Nationality), (Designated only for: US )

Legal Representative:

HARPER David S, McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300  
South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

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Application: WO 2000US14246 20000524 (PCT/WO US0014246)

Priority Application: US 99135481 19990524; US 99140061 19990621

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DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G06T

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 12013

**English Abstract**

Methods and system for general purpose analysis of images acquired from experimental data collected with automated feature-rich, high-throughput experimental data collection systems. A set of pre-determined general assay features is presented. An assay feature includes one or more measurements for an object in a digital photographic image acquired from the experimental data. The set of pre-determined general assay features includes object features, aggregate features and general purpose image processing features. A set of desired assay features is selected from the set of features. A set of images is processed using the desired assay features from the selected set of assay features. The methods and system help provide a general purpose assay development tool. The methods and system allow a biologist, other scientist or lab technician not trained in image processing techniques to quickly and easily design protocols and assays to analyze images acquired from experimental data (e.g., cells). The methods and system may improve the identification, selection,

validation and screening of new drug compounds that have been applied to populations of cells. The methods and system may also be used to provide new bioinformatic techniques to manipulate experimental data including multiple digital photographic images.

#### French Abstract

L'invention concerne des procedes et un systeme pour analyser de maniere polyvalente des images acquises a partir de donnees experimentales recueillies au moyen de systemes automatises de collecte de donnees experimentales presentant de nombreuses caracteristiques et a productivite elevee. L'invention porte egalement sur une serie de caracteristiques d'analyse generales predeterminees. Une caracteristique d'analyse comprend une ou plusieurs mesures pour un objet situe dans une image photographique numerisee obtenue au moyen des donnees experimentales. La serie de caracteristiques d'analyse generales predeterminees comprend les caracteristiques d'objet, d'agregat et de traitement d'images polyvalent. Une serie de caracteristiques d'analyse desirees est choisie parmi la serie de caracteristiques. Une serie d'images est traitee au moyen des caracteristiques d'analyse desirees choisies parmi la serie de caracteristiques d'analyse. Les procedes et le systeme permettent d'obtenir un outil polyvalent de mise au point d'analyses. Les procedes et le systeme permettent a un biologiste, a d'autres scientifiques ou laborantins non qualifies en matiere de techniques de traitement d'images de concevoir rapidement et facilement des protocoles et des analyses permettant d'analyser des images obtenues au moyen des donnees experimentales (par exemple, les cellules). Les procedes et le systeme peuvent ameliorer l'identification, la selection, la validation et le criblage de nouveaux composees pharmaceutiques qui ont ete appliques a des populations de cellules. Ils peuvent egalement fournir de nouvelles techniques bio-informatiques pour manipuler des donnees experimentales, y compris de nombreuses images photographiques numerisees.

Legal Status (Type, Date, Text)

Publication 20001130 A2 Without international search report and to be republished upon receipt of that report.

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6/5/8

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00757878

#### A SYSTEM FOR CELL-BASED SCREENING

#### UN SYSTEME DE CRIBLAGE A BASE DE CELLULES

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US  
(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

OLSON Keith, 372 S. Highland Avenue, Pittsburgh, PA 15205, US, US

(Residence), US (Nationality), (Designated only for: US )

LAPETS Oleg, 1937 Shady Oak Circle, Allison Park, PA 15101, US, US

(Residence), RU (Nationality), (Designated only for: US )

Legal Representative:

HARPER David S, McDonnell, Boehnen, Hulbert & Berghoff, 300 South Wacker Drive, Suite 3200, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200070342 A2 20001123 (WO 0070342)

Application: WO 2000US13208 20000512 (PCT/WO US0013208)

Priority Application: US 99134199 19990514; US 99398965 19990917

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DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

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TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) BR GM HE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM  
Main International Patent Class: G01N-033/50  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 32390

English Abstract

The present invention provides systems, methods, screens, and kits for optical system analysis of cells to rapidly determine the distribution, environment, or activity of luminescently labeled reporter molecules in cells for the purpose of screening large numbers of compounds for those that specifically affect particular biological functions. The invention involves providing cells containing luminescent reporter molecules in an array of locations and scanning numerous cells in each location with a luminescence optical system, converting the optical information into digital data, and utilizing the digital data to determine the distribution, environment or activity of the luminescently labeled reporter molecules in the cells.

French Abstract

La presente invention concerne des systemes, procedes, cribles, et troussees pour un systeme d'analyse optique des cellules afin de determiner rapidement la repartition, l'environnement, ou l'activite de molecules rapporteuses marquees en luminescence dans des cellules en vue du criblage parmi un grand nombre de composees ceux qui presentent un effet particulier sur des fonctions biologiques. L'invention concerne egalement la production de cellules contenant des molecules rapporteuses luminescentes dans un reseau de sites et le balayage de nombreuses cellules dans chacun des sites au moyen d'un systeme optique luminescent, convertissant l'information optique en donnees numeriques, et utilisant les donnees numeriques pour la determination de la repartition, l'environnement ou l'activite des molecules rapporteuses marquees en luminescence dans les cellules.

Legal Status (Type, Date, Text)

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Examination 20010301 Request for preliminary examination prior to end of 19th month from priority date

6/5/9

DIALOG(R)File 349:PCT Fulltext  
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00750319 \*\*Image available\*\*

**ARRANGEMENT FOR EVALUATING FLUORESCENCE-BASED ANALYTICAL REACTIONS**  
**DISPOSITIF D'ANALYSE DE REACTIONS D'IDENTIFICATION A BASE FLUORESCENTE**  
**ANORDNUNG ZUR AUSWERTUNG VON FLUORESZENZBASIERTEN NACHWEISREAKTIONEN**

Patent Applicant/Assignee:

CARL ZEISS JENA GMBH, Carl-Zeiss-Promenade 10, D-07745 Jena, DE, DE  
(Residence), DE (Nationality), (For all designated states except: US)  
CELLOMICSTM INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US  
(Residence), US (Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

BATHE Wolfgang, Neugasse 36, D-07743 Jena, DE, DE (Residence), DE  
(Nationality), (Designated only for: US)  
KUHN Peter, Wollnitzer Strasse 5, D-07745 Jena, DE, DE (Residence), DE  
(Nationality), (Designated only for: US)  
SCHAU Dieter, Dorfstrasse 51, D-07778 Nerkewitz, DE, DE (Residence), DE  
(Nationality), (Designated only for: US)  
FRIEDMAN Alexander L, 1157 Stanton Terrace, Pittsburgh, PA 15201, US, US  
(Residence), US (Nationality), (Designated only for: US)  
GOUGH Albert H, 2014 Louise Drive, Glenshaw, PA 15116-3106, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

CARL ZEISS JENA GMBH, Carl-Zeiss-Promenade 10, D-07745 Jena, DE  
Patent and Priority Information (Country, Number, Date):

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Priority Application: DE 19916748 19990414

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International Patent Class: G01N-035/02

Publication Language: German

Filing Language: German

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 858

#### English Abstract

The invention relates to an arrangement for evaluating fluorescence-based analytical reactions in transparent sample containers. The fluorescence is stimulated and illustrated through the bottom of the container. The samples are inserted from above. An adjustable cover is provided that delimits the sample containers in the upward direction. Said cover is provided with at least one opening for sample insertion. The cover is positioned above the sample containers, which are provided for the insertion, and/or is only open during insertion.

#### French Abstract

Cette invention concerne un dispositif d'analyse de reactions d'identification a base fluorescente dans des recipients transparents. L'activation puis l'apparition de la fluorescence s'effectue a travers le fond du recipient et les echantillons sont introduits par le dessus du recipient. Le dispositif est dote d'un couvercle amovible destine a fermer le dessus du recipient a echantillons, ledit couvercle presentant au moins une ouverture permettant d'introduire les echantillons, et etant place sur le recipient a echantillons a remplir et/ou etant ouvert seulement durant le processus de remplissage.

#### German Abstract

Anordnung zur Auswertung von fluoreszenzbasierten Nachweisreaktionen in durchsichtigen Probengefassen, wobei die Anregung und Abbildung der Fluoreszenz durch den Gefassboden hindurch erfolgt und die Proben von oben eingebracht werden, wobei eine die Probengefasse nach oben abschliessende verstellbare Abdeckung vorgesehen ist, die zur Probeneinfuhrung mindestens eine Offnung aufweist und die nur wahrend des Einfullvorganges oberhalb der zur Einfullung vorgesehenen Probengefasse positioniert ist und/oder nur wahrend des Einfullvorgangs geoffnet ist.

#### Legal Status (Type, Date, Text)

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Search Rpt 20010111 Late publication of international search report  
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6/5/10

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00746984 \*\*Image available\*\*

**MINIATURIZED CELL ARRAY METHODS AND APPARATUS FOR CELL-BASED SCREENING**  
**JEUX ORDONNES D'ECHANTILLONS MINIATURISES ET APPAREIL POUR CRIBLAGE SUR UNE**  
**BASE CELLULAIRE**

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

KAPUR Ravi, 2942 E. Bardoneer Road, Gibsonia, PA 15044, US, IN

(Residence), IN (Nationality), (Designated only for: US)

ADAMS Terri, 100 Eastern Avenue, Pittsburgh, PA 15215, US, US (Residence)  
, US (Nationality), (Designated only for: US)

Legal Representative:

HARPER David S, McDonnell, Boehnen, Hulbert & Berghoff, Suite 3200, 300  
South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

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Application: WO 2000US8767 20000331 (PCT/WO US0008767)

Priority Application: US 99127339 19990401; US 99138119 19990607

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DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ

TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

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Main International Patent Class: G01N-033/543

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Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 34363

English Abstract

The present invention describes novel methods for making a substrate for selective cell patterning, and the substrates themselves, wherein the method comprises contacting reactive hydroxyl groups on the surface of a substrate with a hydroxyl-reactive bifunctional molecule to form a monolayer, and using stencils to deposit cell repulsive or cell adhesive moieties in controlled locations on the cell culture substrate. Methods comprising selective differentiation of stem cells to create tissue specific and organ-specific cell substrates, as well as the cell substrates themselves are also provided.

French Abstract

La presente invention concerne de nouveaux procedes de fabrication de substrat pour une typification selective des cellules, et les substrats eux-memes, dans lesquels ledit procede comprend: la mise en contact de groupes hydroxyles reactifs a la surface d'un substrat avec une molecule bifonctionnelle reactive a l'hydroxyle pour former une monocouche; et au moyen de pochoirs, depot des groupes fonctionnels de cellules de repulsion ou d'adhesion cellulaire dans des sites controles sur le substrat de culture cellulaire. L'invention concerne egalement des procedes comprenant la differenciation selective de cellules embryonnaires pour creer des substrats cellulaires specifiques des tissus et specifiques des organes, ainsi que les substrats cellulaires eux-memes.

Legal Status (Type, Date, Text)

Publication 20001012 A1 With international search report.

Examination 20001214 Request for preliminary examination prior to end of  
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00737885 \*\*Image available\*\*

**A SYSTEM FOR CELL-BASED SCREENING**

**SYSTEME DE CRIBLAGE CELLULAIRE**

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

GIULIANO Kenneth A, 351 Hawthorne Road, Pittsburgh, PA 15209, US, US

(Residence), US (Nationality), (Designated only for: US)

KAPUR Ravi, 2942 E. Bardoneer Road, Gibsonsia, PA 15044, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

HARPER David S (agent), McDonnell, Boehnen, Hulbert & Berghoff, Suite  
3200, 300 South Wacker Drive, Chicago, IL 60606, US,

Patent and Priority Information (Country, Number, Date):

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Application: WO 2000US4794 20000225 (PCT/WO US0004794)

Priority Application: US 99122152 19990226; US 99123399 19990308; US  
99352171 19990712

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DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-015/14

International Patent Class: G01N-033/50; C12M-001/34

Publication Language: English

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Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 46961

English Abstract

The present invention provides systems, methods, screens, reagents and kits for optical system analysis of cells to rapidly determine the distribution, environment, or activity of fluorescently labeled reporter molecules in cells for the purpose of screening large numbers of compounds for those that specifically affect particular biological functions.

French Abstract

La presente invention concerne des systemes, des methodes, des cribles, des reactifs et des kits d'analyse optique de cellules, qui permettent de determiner rapidement la distribution, l'environnement ou l'activite de molecules rapporteurs a marqueur fluorescent. L'objectif recherche est de passer au crible un grand nombre de composes pour localiser ceux d'entre eux qui ont une incidence specifique sur des fonctions biologiques particulieres.

Legal Status (Type, Date, Text)

Publication 20000831 A2 Without international search report and to be republished upon receipt of that report.

Examination 20000928 Request for preliminary examination prior to end of 19th month from priority date

Search Rpt 20010308 Late publication of international search report

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6/5/12

DIALOG(R) File 349:PCT Fulltext

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00736205 \*\*Image available\*\*

METHOD AND SYSTEM FOR DYNAMIC STORAGE RETRIEVAL AND ANALYSIS OF  
EXPERIMENTAL DATA WITH DETERMINED RELATIONSHIPS  
PROCEDE ET SYSTEME DE RECHERCHE DYNAMIQUE DANS UNE MEMOIRE ET ANALYSE DE  
DONNEES EXPERIMENTALES A RELATIONS DETERMINEES

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US  
(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

WANG Jian, 401 Shady Avenue, Pittsburgh, PA 15206, US, US (Residence), CN  
(Nationality), (Designated only for: US)

HARRINGTON Christopher C, 4 Edgewood Road, Pittsburgh, PA 15206, US, US

(Residence), US (Nationality), (Designated only for: US)  
TAYLOR D Lansing, 910 Notre Dame Place, Pittsburgh, PA 15215, US  
(Residence), US (Nationality), (Designated only for: US)  
QU Long, 2616 Patrice Court, Murrys ville, PA 15668, US, US (Residence),  
CN (Nationality), (Designated only for: US)

Legal Representative:

LESAVICH Stephen, McDonnell, Boehnen, Hulbert & Berghoff, Suite 3200, 300  
South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200049540 A1 20000824 (WO 0049540)

Application: WO 2000US4331 20000218 (PCT/WO US0004331)

Priority Application: US 99120801 19990219

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK

DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ

TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G06F-017/50

International Patent Class: G06F-017/30; G06F-019/00 -159:00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 14206

English Abstract

A method and system for dynamic storage, retrieval and display of experimental information with determined relationships. A graphical user interface is presented from which shapes and arrows representing biological entities and transformations respectively, can be input and edited. Multidimensional information based on a pre-determined hierarchy is input to link the entities and transformations to additional information about the entities and transformations. Related information, if any, is input to link the entities and transformations to other information in plural external databases on a public network such as the Internet. Information associated with plural shapes connected with plural arrows is saved as a biological pathway with determined relationships in a database. The biological pathway defines a hierarchical representation of a biological function with determined relationships between entities and transformations. Biological pathway diagrams such as cell pathways with determined relationships may be dynamically input, edited and dynamically generated to represent biological functions, such as cellular functions, to enable a user to visually interact with identified dimensions of biological information. A user may dynamically navigate through identified dimensions of biological information to find out a relationship of a specific piece of biological information with other pieces of biological information. The method and system may help facilitate the abstraction of knowledge from information for biological pathways and provide new bioinformatic techniques.

French Abstract

L'invention concerne un procede et un systeme de stockage, de recherche et d'affichage dynamiques de donnees experimentales a relations determinees. Une interface utilisateur graphique est presentee, a partir de laquelle des formes et des fleches representant respectivement des entites biologiques et des transformations, peuvent etre introduites ou modifiees. Des donnees multidimensionnelles basees sur une hierarchie predeterminee sont introduites afin de relier les entites et les transformations aux donnees additionnelles relatives aux entites et aux transformations. Les donnees relatives, s'il en existe, sont introduites afin de relier les entites et les transformations a d'autres donnees de plusieurs bases de donnees exterieures sur un reseau public, tel qu'Internet. Les donnees associees a diverses formes reliees avec plusieurs fleches sont sauvegardees dans une base de donnees en tant que voie biologique comportant des relations determinees. La voie biologique

definit une representation hierarchique d'une fonction biologique comportant des relations determinees entre les entites et les transformations. Les diagrammes de la voie biologique, tels que des voies cellulaires comportant des relations determinees peuvent etre introduits, modifies et generes de maniere dynamique pour représenter des fonctions biologiques, telles que des fonctions cellulaires, de facon a permettre a un utilisateur d'agir visuellement sur des dimensions identifiees des donnees biologiques. Un utilisateur peut explorer de maniere dynamique des dimensions identifiees des donnees biologiques pour decouvrir une relation de donnees biologiques specifiques avec d'autres donnees biologiques. Le procede et le systeme peuvent contribuer a faciliter l'abstraction de connaissances a partir de donnees relatives aux voies biologiques, et fournir de nouvelles techniques bioinformatiques.

Legal Status (Type, Date, Text)

Publication 20000824 A1 With international search report.

Publication 20000824 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Examination 20001116 Request for preliminary examination prior to end of 19th month from priority date

6/5/13

DIALOG(R) File 349:PCT Fulltext

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00717089 \*\*Image available\*\*

**METHODS AND SYSTEM FOR EFFICIENT COLLECTION AND STORAGE OF EXPERIMENTAL DATA**

**PROCEDES ET SYSTEME DE COLLECTION ET DE STOCKAGE EFFICACES DE DONNEES EXPERIMENTALES**

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15328, US, US  
(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

BOYCE Keith S, 2589 Cole Road, Wexford, PA 15090, US, US (Residence), US  
(Nationality), (Designated only for: US)

MCKENNA Brian K, 224 Sleepy Hollow Road, Pittsburgh, PA 15216, US, US  
(Residence), US (Nationality), (Designated only for: US)

GLICK Phillip W, 1022 Shawnee Ridge Drive, Cheswick, PA 15024, US, US  
(Residence), US (Nationality), (Designated only for: US)

DUNLAY Terry R, 433 Lynn Ann Drive, Plum Boro, PA 15068, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

LESAVICH Stephen, McDonnell, Boehnen, Hulbert & Berghoff, Suite 3200, 300  
South Wacker Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200029984 A2 20000525 (WO 0029984)

Application: WO 99US26562 19991110 (PCT/WO US9926562)

Priority Application: US 98108291 19981113; US 98110643 19981201; US  
99140240 19990621; US 99142375 19990706; US 99142646 19990706

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE  
ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT  
UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G06F-017/30

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 15807

English Abstract



Methods and system for efficient collection and storage of experimental data. These methods and system allow experimental data from high-throughput, feature-rich data collection systems, such as high-throughput cell data collection systems to be efficiently collected, stored, managed and displayed. The methods and system can be used, for example, for storing managing and displaying cell image data and cell feature data collected from microplates including multiple wells and a variety of bio-chips in which an experimental compound has been applied to a population of cells. The methods and system provide a flexible and scalable repository of experimental data including multiple databases at multiple locations including pass-through databases that can be easily managed and allows cell data to be analyzed, manipulated and archived. The methods and system may improve the identification, selection, validation and screening of new drug compounds that have been applied to populations of cells. The methods and system can also be used to provide new bioinformatic techniques to manipulate experimental data including multiple digital photographic images.

#### French Abstract

L'invention concerne des procedes et systeme de collection et de stockage de donnees experimentales. Ces procedes et systeme permettent de collecter, stocker, gerer et afficher de maniere efficace des donnees experimentales provenant de systemes de collection de donnees de fonction riches a productivite elevee, tels que des systemes de donnees de cellules a productivite elevee. Par exemple, ils peuvent etre utilises pour stocker, gerer et afficher des donnees d'image de cellules et des donnees de fonction de cellules recueillies de microplaques comprenant plusieurs puits et une variete de bio-puces dans lesquelles un compose experimental a ete applique a une population de cellules. Lesdits procedes et systeme fournissent un repertoire souple et a l'echelle de donnees experimentales comprenant plusieurs bases de donnees a de nombreux emplacements comportant des bases de donnees de passage aisement gerables et permettent aux donnees de cellules d'etre analysees, manipulees et archivees. Lesdits procedes et systeme peuvent ameliorer l'identification, la selection, la validation et le criblage de composes de medicaments nouveaux qui ont ete appliques a des populations de cellules. Ils peuvent egalement servir a l'apport de techniques bio-informatiques nouvelles pour manipuler des donnees experimentales renfermant de nombreuses images photographiques numerisees.

#### Legal Status (Type, Date, Text)

Publication 20000525 A2 Without international search report and to be republished upon receipt of that report.  
Examination 20000817 Request for preliminary examination prior to end of 19th month from priority date  
Search Rpt 20001130 Late publication of international search report

6/5/14

DIALOG(R) File 349:PCT Fulltext

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00713629

#### A SYSTEM FOR CELL-BASED SCREENING

#### SYSTEME DE CRIBLAGE POUR CELLULES

Patent Applicant/Assignee:

CELLOMICS INC, 635 William Pitt Way, Pittsburgh, PA 15238, US, US

(Residence), US (Nationality), (For all designated states except: US

Patent Applicant/Inventor:

GUILIANO Kenneth A, 351 Hawthorne Road, Pittsburgh, PA 15209, US, US

(Residence), US (Nationality), (Designated only for: US)

BRIGHT Gary, 586 Sturbridge Drive, Highland Heights, OH 44143, US, US

(Residence), US (Nationality), (Designated only for: US)

OLSON Keith, 372 S. Highland Avenue, Pittsburgh, PA 15205, US, US

(Residence), US (Nationality), (Designated only for: US)

BURROUGHS-TENCZA Sarah, 401 Olympia Road, Pittsburgh, PA 15211, US, US

(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

HALLORAN Patrick J, McDonnell, Boehnen, Hulbert & Berghoff, Suite 3200,

300 South Wacker Drive, Chicago, IL 60606, US  
Patent and Priority Information (Country, Number, Date):  
Patent: WO 200026408 A2 20000511 (WO 0026408)  
Application: WO 99US25431 19991029 (PCT/WO US9925431)  
Priority Application: US 98106308 19981030; US 99136078 19990526  
Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE  
ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT  
UA UG US UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12Q-001/68

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 41645

#### English Abstract

The present invention provides systems, methods, screens, reagents and kits for optical system analysis of cells to rapidly determine the distribution, environment, or activity of fluorescently labeled reporter molecules in cells for the purpose of screening large numbers of compounds for those that specifically affect particular biological functions.

#### French Abstract

La presente invention concerne des systemes, des methodes, des cribles, des reactifs et des kits d'analyse optique de cellules, qui permettent de determiner rapidement la distribution, l'environnement ou l'activite de proteines rapporteurs a marqueur fluorescent. L'objectif recherche est de passer au crible un grand nombre de composes pour localiser ceux d'entre eux qui ont une incidence specifique sur des fonctions biologiques particulieres.

#### Legal Status (Type, Date, Text)

Publication 20000511 A2 Without international search report and to be republished upon receipt of that report.  
Examination 20000706 Request for preliminary examination prior to end of 19th month from priority date  
Search Rpt 20000914 Late publication of international search report

6/5/15

DIALOG(R)File 349:PCT Fulltext

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00704226 \*\*Image available\*\*

**A SYSTEM FOR CELL-BASED SCREENING**

**SYSTEME DE CRIBLAGE CELLULAIRE**

Patent Applicant/Assignee:

CELLOMICS INC, CELLOMICS, INC. , 635 William Pitt Way, Pittsburgh, PA  
15238 , US

Inventor(s):

DUNLAY R Terry, DUNLAY, R., Terry , 433 Lynn Ann Drive, New Kensington,  
PA 15068 , US

TAYLOR D Lansing, TAYLOR, D., Lansing , 910 Notre Dame Place, Pittsburgh,  
PA 15215 , US

GOUGH Albert H, GOUGH, Albert, H. , 635 William Pitt Way, Pittsburgh, PA  
15238 , US

GUILIANO Kenneth A, GUILIANO, Kenneth, A. , 2014 Louise Drive, Glenshaw,  
PA 15116 , US

RUBIN Richard A, RUBIN, Richard, A. , 5866 Bartlett Street, Pittsburgh,  
PA 15217 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0017643 A2 20000330 (WO 200017643)

Application: WO 99US21561 19990917 (PCT/WO US9921561)  
Priority Application: US 98100973 19980918  
Designated States: AU CA JP MX NZ US AT BE CH CY DE DK ES FI FR GB GR IE IT  
LU MC NL PT SE  
Main International Patent Class: G01N-033/53;  
International Patent Class: G01N-015/14;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 32399

#### English Abstract

The present invention provides systems, methods, screens, and kits for optical system analysis of cells to rapidly determine the distribution, environment, or activity of fluorescently labeled reporter molecules in cells for the purpose of screening large numbers of compounds for those that specifically affect particular biological functions. The invention involves providing cells containing fluorescent reporter molecules in an array of locations and scanning numerous cells in each location with a high magnification fluorescence optical system, converting the optical information into digital data, and utilizing the digital data to determine the distribution, environment or activity of the fluorescently labeled reporter molecules in the cells. The array of locations may be an industry standard 96 well or 384 well microtiter plate or a microplate which is a microplate having cells in a micropatterned array of locations. The invention includes apparatus and computerized method for processing, displaying and storing the data.

#### French Abstract

La presente invention concerne des systemes, des procedes, des cribles et des kits destines a l'analyse par systeme optique de cellules afin de determiner rapidement la distribution, l'environnement, ou l'activite de molecules reporters a marquage fluorescent dans des cellules en vue du criblage d'un grand nombre de composes, criblage destine a determiner lesquels de ces composes agissent de maniere specifique sur des fonctions biologiques particulieres. Le procede selon l'invention consiste a utiliser des cellules renfermant des molecules reporters a marquage fluorescent dans un reseau d'emplacements; a proceder au balayage optique de nombreuses cellules dans chaque emplacement a l'aide d'un systeme optique fluorometrique de grossissement; a convertir les informations optiques en donnees numeriques; et a utiliser ces donnees numeriques pour determiner la distribution, l'environnement ou l'activite des molecules reporters a marquage fluorescent. Le reseau d'emplacements peut etre une plaque de microtitrage standard a 96 ou 384 puits, telles que celles que l'on trouve dans l'industrie, ou une microplaque portant des cellules disposees dans un reseau d'emplacements microstructure. L'invention concerne egalement un appareil et un procede informatique permettant de traiter, d'afficher et de stocker ces donnees.

#### Legal Status (Type, Date, Text)

Examination 20000615 Request for preliminary examination prior to end of  
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Correction 20000622 Corrections of entry in Section 1: delete "(63)"  
Correction 20000817 Corrected version of Pamphlet: pages 1/24-24/24,  
drawings, replaced by new pages 1/24-24/24; due to  
late transmittal by the receiving Office  
Search Rpt 20001012 Late publication of international search report

6/5/16

DIALOG(R)File 349:PCT Fulltext  
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00704213 \*\*Image available\*\*

MINIATURIZED CELL ARRAY METHODS AND APPARATUS FOR CELL-BASED SCREENING  
METHODES ET APPAREIL DE CRIBLAGE SUR LA BASE DE CELLULES AVEC UN RESEAU  
MINIATURISE DE CELLULES

Patent Applicant/Assignee:

CELLOMICS INC, CELLOMICS, INC. , 635 William Pitt Way, Pittsburgh, PA  
15238 , US

Inventor(s):

JUNG David R, JUNG, David, R. , 122 Crescent Hills Road, Pittsburgh, PA  
15235 , US

GOUGH Albert H, GOUGH, Albert, H. , 2014 Louise Drive, Glenshaw, PA 15116  
US

TAYLOR D Lansing, TAYLOR, D., Lansing , 910 Notre Dame Place, Pittsburgh,  
PA 15215 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0017624 A2 20000330 (WO 200017624)

Application: WO 99US21729 19990922 (PCT/WO US9921729)

Priority Application: US 98101399 19980922

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE  
ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT  
UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ TZ UG ZW AM AZ BY KG KZ  
MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ  
CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: G01N-015/14;

International Patent Class: G01N-033/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 24574

English Abstract

The present invention devices and methods for maximizing the number of wells that can be imaged at one time while still obtaining adequate pixel resolution in the image. This result has been achieved through the use of fluidic architectures that maximizes well density. The present invention thus provides a miniaturized microplate system with closed fluidic volumes that are internally supplied with fluid exchange, and with wells that are closely spaced to more rapidly detect spatially-resolved features of individual cells.

French Abstract

La presente invention concerne des dispositifs et methodes permettant d'accroitre le nombre de cupules pouvant etre representees en une seule fois tout en realisant une definition de pixels appropriee dans l'image. On a obtenu ce resultat grace a l'utilisation d'architectures fluidiques qui accroissent la densite des cupules. La presente invention concerne donc un systeme de microplaques miniaturisees comportant des volumes fluidiques fermes alimentes de facon interne par echange de fluides, et des cupules rapprochees pour une detection rapide de caracteristiques a definition spatiale de cellules individuelles.

Legal Status (Type, Date, Text)

Examination 20000608 Request for preliminary examination prior to end of  
19th month from priority date

Search Rpt 20010104 Late publication of international search report

6/5/17

DIALOG(R)File 349:PCT Fulltext

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00690104

**A SYSTEM FOR CELL-BASED SCREENING**

**SYSTEME DESTINE A UN CRIBLAGE A BASE DE CELLULES**

Patent Applicant/Assignee:

CELLOMICS INC, CELLOMICS, INC. , 635 William Pitt Way, Pittsburgh, PA  
15238 , US

Inventor(s):

RUBIN Richard A, RUBIN, Richard, A. , 35 William Pitt Way, Pittsburgh, PA  
15238 , US

GIULIANO Ken A, GIULIANO, Ken, A. , 635 William Pitt Way, Pittsburgh, PA

15238 , US

GOUGH Albert, GOUGH, Albert , 635 William Pitt Way, Pittsburgh, PA 15238  
, US

DUNLAY Terry, DUNLAY, Terry , 635 William Pitt Way, Pittsburgh, PA 15238  
, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0003246 A2 20000120 (WO 200003246)

Application: WO 99US15870 19990713 (PCT/WO US9915870)

Priority Application: US 9892671 19980713

Designated States: AU CA JP MX US AT BE CH CY DE DK ES FI FR GB GR IE IT LU  
MC NL PT SE

Main International Patent Class: G01N-033/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 31559

English Abstract

The present invention provides systems, methods, and screens to measure receptor internalization in a single step with appropriate automation and throughput. This approach involves luminescent labeling of the receptor of interest and the automated measurement of receptor internalization to a perinuclear location.

French Abstract

La presente invention concerne des systemes, des procedes et des techniques de criblage qui servent a mesurer l'internalisation a un stade individuel avec une automatisation et un debit appropries. Les procedes et techniques de l'invention necessitent le marquage luminescent du recepteur d'interet et la mesure automatisee de l'internalisation de recepteurs vers un emplacement perinucleaire.

Legal Status (Type, Date, Text)

Correction 20000720 Corrected version of Pamphlet: pages 1/30-30/30,  
drawings, replaced by new pages 1/30-30/30; due to  
late transmittal by the receiving Office

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18apr01 15:41:28 User026066 Session D6404.3

Sub account: 2087-010140

\$8.60 1.812 DialUnits File349

\$275.40 54 Type(s) in Format 5

\$275.40 54 Types

\$284.00 Estimated cost File349

\$0.79 TELNET

\$284.79 Estimated cost this search

\$284.79 Estimated total session cost 1.812 DialUnits

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DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 00.12.12D

Last logoff: 02may01 14:37:41

Logon file001 02may01 16:25:25

NOTICE set ON to \$20.00

You will be prompted to confirm each TYPE or PRINT request where  
format charges exceeds \$20.00

Please enter SUBACCOUNT name/number:

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Is 3776-010140 LAUNCHCYTE BEJ the SUBACCOUNT you want to use? (Y/N)

?y

Subaccount is set to 3776-010140 LAUNCHCYTE BEJ

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File 1:ERIC 1966-2001/Apr 17

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Set Items Description

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Terminal set to DLINK

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\$0.21 Estimated cost File1

\$0.07 TELNET

\$0.28 Estimated cost this search

\$0.28 Estimated total session cost 0.060 DialUnits

File 349:PCT Fulltext 1983-2001/UB=20010419, UT=20010405

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Set Items Description

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E3	171	*AU=SCHREIBER
E4	1	AU=SCHREIBER ACHIM
E5	5	AU=SCHREIBER ALAN D
E6	1	AU=SCHREIBER ALFRED
E7	2	AU=SCHREIBER BERND
E8	1	AU=SCHREIBER BIRGIT
E9	1	AU=SCHREIBER BRIAN E
E10	1	AU=SCHREIBER CHRISTOPHER M
E11	1	AU=SCHREIBER DANIEL
E12	1	AU=SCHREIBER DANIEL JAMES

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E4	5	AU=SCHREIBER THOMAS
E5	3	AU=SCHREIBER WERNER
E6	1	AU=SCHREIBER WIGBERT G
E7	4	AU=SCHREIBER WILLIAM F
E8	1	AU=SCHREIBER WILLIAM L
E9	4	AU=SCHREIBER WOLFGANG
E10	1	AU=SCHREIBER YANNICK
E11	1	AU=SCHREIBER ZVI
E12	2	AU=SCHREIBER-AVISSAR SOFIA

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E3	15	*AU=MACBEATH
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E5	8	AU=MACBETH
E6	1	AU=MACBETH FIONA SUSAN
E7	1	AU=MACBETH IAN
E8	5	AU=MACBETH KYLE J
E9	1	AU=MACCABA
E10	1	AU=MACCABA BRIAN
E11	2	AU=MACCABEE
E12	1	AU=MACCABEE JOHN

Enter P or PAGE for more

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S1 16 AU=SCHREIBER STUART L

?t 1/51/

1/51/1

>>>Format 51 is not valid in file 349

?t 1/5/1

1/5/1

DIALOG(R)File 349:PCT Fulltext

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00715225 \*\*Image available\*\*

**FK506-BASED REGULATION OF BIOLOGICAL EVENTS**

**REGULATION FONDEE SUR FK506 D'EVENEMENTS BIOLOGIQUES**

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, 124 Mount Auburn Street,  
Cambridge, MA 02138-5701, US, US (Residence), US (Nationality), (For  
all designated states except: US)

Patent Applicant/Inventor:

CLEMONS Paul A, 23 Elm Street #109, Somerville, 02143, US, US (Residence)  
, US (Nationality), (Designated only for: US )

GLADSTONE Brian G, 50 Harbor Point Boulevard #208, Dorchester, MA 02125,  
US, US (Residence), GB (Nationality), (Designated only for: US )

SETH Abhinav, 300 Engle Street, Tenafly, NJ 07670, US, US (Residence), US  
(Nationality), (Designated only for: US )

SCHREIBER Stuart L , 434 Marlborough Street, Boston, MA 02115, US, US  
(Residence), US (Nationality), (Designated only for: US

Legal Representative:

HAUSDORFF Sharon F, Ariad Pharmaceuticals, Inc., 26 Landsdowne Street,  
Cambridge, MA 02139-4234, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200028011 A2 20000518 (WO 0028011)

Application: WO 99US25766 19991105 (PCT/WO US9925766)

Priority Application: US 98107473 19981106

Designated States: AU CA JP US  
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Main International Patent Class: C12N-015/00  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 39109

English Abstract

Materials and methods are disclosed for regulation of biological events such as target gene transcription and growth, proliferation or differentiation of engineered cells.

French Abstract

On decrit des matieres et des procedes qui permettent de reguler des evenements biologiques tels que la transcription de genes cibles et la croissance, la proliferation ou la differenciation de cellules genetiquement modifiees.

Legal Status (Type, Date, Text)

Publication 20000518 A2 Without international search report and to be republished upon receipt of that report.  
Examination 20000713 Request for preliminary examination prior to end of 19th month from priority date  
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?y

1/5/2

DIALOG(R)File 349:PCT Fulltext  
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00693858 \*\*Image available\*\*

**METHOD OF HIGH-THROUGHPUT SCREENING OF MOLECULES AND COMPOUNDS FOR THEIR EFFECTS ON BIOLOGICAL AND CHEMICAL PROCESSES**  
**PROCEDE D'EXAMEN HAUTE EFFICACITE DE MOLECULES ET DE COMPOSES EN RAPPORT AVEC LEURS EFFETS SUR DES PROCESSUS BIOLOGIQUES ET CHIMIQUES**

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, PRESIDENT AND FELLOWS OF HARVARD COLLEGE, 17 Quincy Street, Cambridge, MA 02138, US

Inventor(s):

STOCKWELL Brent R, STOCKWELL, Brent, R., Apartment 6, 21 Beacon Street, Boston, MA 02108, US

SCHREIBER Stuart L, SCHREIBER, Stuart, L., 434 Marlborough Street, Boston, MA 02108, US

HAGGARTY Stephen J, HAGGARTY, Stephen, J., 5 Arnold Court, Somerville, MA 02143, US

MITCHISON Timothy J, MITCHISON, Timothy, J., 87 Fuller Street, Brookline, MA 02146, US

KAPOOR Tarun M, KAPOOR, Tarun, M., Apartment 18, 22 Chauncy Street, Cambridge, MA 02138, US

MAYER Thomas, MAYER, Thomas, 93 Thorndike Street, Brookline, MA 02446, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0007017 A2 20000210 (WO 200007017)

Application: WO 99US17046 19990727 (PCT/WO US9917046)

Priority Application: US 9894305 19980727; US 99131765 19990430; US 99137039 19990601

Designated States: AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: G01N-033/53;

Publication Language: English

Filing Language: English

Fulltext Availability:



Detailed Description  
Claims  
Fulltext Word Count: 28392

English Abstract

The present invention provides a system for high-throughput analysis of chemical compounds. Assays are performed in a high density platform, and compounds having pre-determined desirable effects are identified. Preferably, the compounds have biological effects, more preferably, the assays and detection are performed on whole cells.

French Abstract

L'invention concerne un systeme permettant d'effectuer une analyse hautement efficace de composés chimiques. Les examens, effectués sur une plate-forme haute densité, permettent d'identifier les composés exerçant des effets désirables prédéterminés. De préférence, les composés possèdent des effets biologiques, et les examens et la détection sont si possible effectués sur des cellules entières.

Legal Status (Type, Date, Text)

Examination 20001221 Request for preliminary examination prior to end of 19th month from priority date

1/5/3

DIALOG(R) File 349:PCT Fulltext  
(c) 2001 WIPO/MicroPat. All rts. reserv.

00693524 \*\*Image available\*\*

SYNTHESIS OF COMBINATORIAL LIBRARIES OF COMPOUNDS REMINISCENT OF NATURAL PRODUCTS

SYNTHESE COMBINATOIRE D'ECHANTILLOTHEQUES DE COMPOSES REMINISCENTS DE PRODUITS NATURELS

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, PRESIDENT AND FELLOWS OF HARVARD COLLEGE, 17 Quincy Street, Cambridge, MA 02138, US

Inventor(s):

SCHREIBER Stuart L, SCHREIBER, Stuart, L., 434 Marlborough Street, Boston, MA 02110, US

SHAIR Matthew D, SHAIR, Matthew, D., Apartment 2, 16 Foskett Street, Somerville, MA 02144, US

TAN Derek S, TAN, Derek, S., 437 True Hickory Drive, Rochester, NY 14615-1321, US

FOLEY Michael A, FOLEY, Michael, A., 107 Conwell Avenue, Somerville, MA 02144, US

STOCKWELL Brent R, STOCKWELL, Brent, R., Apartment 6R, 21 Beacon Street, Boston, MA 02108, US

STERNSON Scott M, STERNSON, Scott, M., 464 Margo Lane, Berwyn, PA 19312, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 0006525 A2 20000210 (WO 200006525)

Application: WO 99US16753 19990722 (PCT/WO US9916753)

Priority Application: US 98121922 19980725

Designated States: AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: C07B-061/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 70794

English Abstract

The present invention provides complex compounds reminiscent of natural products and libraries thereof, as well as methods for their production. The inventive compounds and libraries of compounds are reminiscent of natural products in that they contain one or more stereocenters, and a high density and diversity of functionality. In general, the inventive libraries are synthesized from diversifiable scaffold structures, which

are synthesized from readily available or easily synthesizable template structures. In certain embodiments, the inventive compounds and libraries are generated from diversifiable scaffolds synthesized from a shikimic acid based epoxyol template. In other embodiments, the inventive compounds and libraries are generated from diversifiable scaffolds synthesized from the pyridine-based template isonicotinamide. The present invention also provides a novel ortho-nitrobenzyl photolinker and a method for its synthesis. Furthermore, the present invention provides methods and kits for determining one or more biological activities of members of the inventive libraries. Additionally, the present invention provides pharmaceutical compositions containing one or more library members.

#### French Abstract

La presente invention concerne, d'une part des composés complexes reminiscents de produits naturels ainsi que des échantillons de ces composés, et d'autre part des procédés de production correspondants. Ces composés et échantillons de composés sont des reminiscences de produits naturels en ce qu'ils contiennent au moins un centre stéréotaxique et présentent des fonctionnalisations très denses et diverses. En général, la synthèse des échantillons de l'invention se fait à partir d'ossatures d'échaffaudages diversifiables dont la synthèse se fait à partir de structures gabarit existantes ou facilement synthétisables. Pour certaines réalisations, les composés et échantillons de l'invention sont générés à partir d'échaffaudages diversifiables synthétisés à partir d'un gabarit epoxyol à base d'acide shikimique. Pour d'autres réalisations, les composés et échantillons de l'invention sont générés à partir d'échaffaudages diversifiables synthétisés à partir d'un gabarit isonicotinamide à base de pyridine. L'invention concerne également un photoréticulant à base d'orthonitrobenzyle ainsi que le procédé de synthèse approprié. En outre, l'invention concerne des procédés ainsi que des nécessaires permettant de déterminer une ou plusieurs activités biologiques des éléments des échantillons de l'invention. Enfin, l'invention concerne des compositions pharmaceutiques contenant l'un au moins des éléments de bibliothèque.

Legal Status (Type, Date, Text)

Examination 20000713 Request for preliminary examination prior to end of 19th month from priority date

Search Rpt 20001116 Late publication of international search report

1/5/4

DIALOG(R) File 349:PCT Fulltext

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00657644

#### NOVEL DIMERIZING AGENTS, THEIR PRODUCTION AND USE

#### AGENTS DE DIMERISATION, PRODUCTION ET UTILISATION

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, PRESIDENT AND FELLOWS OF HARVARD COLLEGE, 124 Mount Auburn Street, Cambridge, MA 02138, US

Inventor(s):

SCHREIBER Stuart L, SCHREIBER, Stuart, L., 434 Marlborough Street, Boston, MA 02115, US

CRABTREE Gerald R, CRABTREE, Gerald, R., 7 Durham Road, Woodside, CA 94062, US

LIBERLES Stephen D, LIBERLES, Stephen, D., 68R Dane Street, Somerville, MA 02143, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9941258 A1 19990819

Application: WO 99US3095 19990212 (PCT/WO US9903095)

Priority Application: US 9874584 19980213

Designated States: AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: C07D-498/18;

International Patent Class: C07K-001/107; C07K-001/14; C12N-015/56;

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C07D-311/00; C07D-273/00; C07D-209/00;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 59294

English Abstract

Materials and methods are disclosed for regulation of biological events such as target gene transcription and growth, proliferation or differentiation of engineered cells.

French Abstract

L'invention concerne des agents et des procedes permettant de réguler un certain nombre d'évenements biologiques, comme la transcription et la croissance de genes cibles, ou la proliferation et la differenciation de cellules manipulees.

1/5/5

DIALOG(R)File 349:PCT Fulltext  
(c) 2001 WIPO/MicroPat. All rts. reserv.

00571602

**DROPLET ASSAY SYSTEM**

**SYSTEME DE CRIBLAGE PAR GOUTTELETTES**

Patent Applicant/Assignee:

THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE, THE PRESIDENT AND FELLOWS  
OF HARVARD COLLEGE, 17 Quincy Street, Cambridge, MA 02138, US

Inventor(s):

SCHREIBER Stuart L , SCHREIBER, Stuart, L., 434 Marlborough Street,  
Boston, MA 02116, US

SHAIR Matthew D, SHAIR, Matthew, D., Apartment 2, 16 Foskett Street,  
Somerville, MA 02144, US

BORCHARDT Allen J, BORCHARDT, Allen, J., Apartment 14, 2 Arlington  
Street, Cambridge, MA 02138, US

YOU Angie J, YOU, Angie, J., 498 Adams House, Cambridge, MA 02138, US

HUANG Jing, HUANG, Jing, Apartment 2, 66 Z Prentiss Street, Cambridge, MA  
02140, US

FOLEY Mike, FOLEY, Mike, 107 Conwell Avenue, Somerville, MA 02144, US

TAN Derek, TAN, Derek, 23 Berkeley Street, Cambridge, MA 02138, US

WHITESIDES George, WHITESIDES, George, 124 Grasmere Street, Newton, MA  
02158, US

JACKMAN Rebecca J, JACKMAN, Rebecca, J., Apartment 2, 38 Groves Street,  
Boston, MA 02114, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9816830 A2 19980423

Application: WO 97US19110 19971015 (PCT/WO US9719110)

Priority Application: US 9629128 19961016; US 9749864 19970606

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW  
MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN GH KE LS MW  
SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE  
IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Main International Patent Class: G01N-033/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Fulltext Word Count: 23798

English Abstract

The present invention provides a novel system for identifying compounds having desirable chemical or biological activities. According to the invention, test compounds are introduced into liquid droplets and assayed therein. The system is particularly useful for identifying compounds that act e.g., as catalysts, or that have biological activities. In preferred embodiments of the invention, the compounds are assayed < i> in vivo< /i>

French Abstract

L'invention concerne un nouveau systeme permettant d'identifier des composes ayant des activites chimiques ou biologiques desirables. Selon l'invention, on introduit des composes a etudier dans des gouttelettes de liquide et on les crible. Le systeme est particulierement utile pour identifier des composes qui agissent par exemple comme catalyseurs ou qui ont des activites biologiques. Dans les modes de realisation preferes de l'invention, les composes sont cribles in vivo.

1/5/6

DIALOG(R)File 349:PCT Fulltext

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00569860

REACTIVE LIGANDS AND COVALENT LIGAND;ndash;PROTEIN COMPLEXES

LIGANDS REACTIFS ET COMPLEXES COVALENTS DE LIGANDS ET PROTEINES

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, PRESIDENT AND FELLOWS OF  
HARVARD COLLEGE, 17 Quincy Street, Cambridge, MA 02138, US

Inventor(s):

WILEY Don C, WILEY, Don, C., &ndash;;, US

SCHREIBER Stuart L , SCHREIBER, Stuart, L., &ndash;;, US

VALENTEKOVICH Robert J, VALENTEKOVICH, Robert, J., &ndash;;, US

WEISS Gregory A, WEISS, Gregory, A., &ndash;;, US

SHAMBAYATI Soroosh, SHAMBAYATI, Soroosh, &ndash;;, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9814208 A1 19980409

Application: WO 97US17483 19970930 (PCT/WO US9717483)

Priority Application: US 9627081 19960930

Designated States: JP US AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: A61K-039/00;

International Patent Class: C07K-007/06;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 9473

English Abstract

Ligands bind to proteins according to particular kinetic profiles. The reversible kinetics of binding lead to release of the ligand from the binding site, thereby affecting the recognition of the ligand;ndash; protein complex. By changing the kinetics of the reversible binding, it is possible to alter the response to the cellular immune system. Reactive ligands can be used to change the kinetics of binding by reacting with the protein when bound.

French Abstract

Des ligands se lient a des proteines conformement a certains profils cinetiques particuliers. La cinetique de liaison reversible entraine la liberation du ligand du site de liaison, ce qui modifie la capacite de reconnaissance du complexe ligand;ndash;proteine. En modifiant la cinetique de la liaison reversible, il est possible de modifier la reaction face au systeme immunitaire cellulaire. On peut utiliser, pour modifier la cinetique de liaison, des ligands reactifs qui reagissent avec la proteine une fois la liaison formee.

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DIALOG(R)File 349:PCT Fulltext

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00564605

\*\*Image available\*\*

USE OF MUTATED MACROLIDE BINDING PROTEIN FOR THE PREVENTION OF GUHD

UTILISATION D'UNE PROTEINE DE FIXATION DE MACROLIDE MUTEE POUR PREVENIR LA

**REACTION DU GREFFON CONTRE L'HOTE**

**Patent Applicant/Assignee:**

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, PRESIDENT AND FELLOWS OF  
HARVARD COLLEGE, 17 Quincy Street, Cambridge, MA 02138, US  
THE LELAND S STANFORD JUNIOR UNIVERSITY, THE LELAND S. STANFORD JUNIOR  
UNIVERSITY, Office of Technology Licensing, Suite 350, 900 Welch Road,  
Palo Alto, CA 94304, US  
SCHREIBER Stuart L  
BELSHAW Peter J  
CRABTREE Gerald

**Inventor(s):**

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, PRESIDENT AND FELLOWS OF  
HARVARD COLLEGE, 17 Quincy Street, Cambridge, MA 02138, US  
THE LELAND S STANFORD JUNIOR UNIVERSITY, THE LELAND S. STANFORD JUNIOR  
UNIVERSITY, Office of Technology Licensing, Suite 350, 900 Welch Road,  
Palo Alto, CA 94304, US  
SCHREIBER Stuart L  
BELSHAW Peter J  
CRABTREE Gerald

**Patent and Priority Information (Country, Number, Date):**

Patent: WO 9808956 A2 19980305  
Application: WO 97US15153 19970827 (PCT/WO US9715153)  
Priority Application: US 9624666 19960827

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FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW  
MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN GH KE LS MW  
SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE  
IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Main International Patent Class: C12N-015/54;

International Patent Class: C12N-015/12; C12N-005/10; C12N-009/12;  
C07K-014/715; A61K-048/00; A01K-067/027;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 32008

**English Abstract**

This invention is directed to a modified cyclosporin A and to a modified, genetically engineered version of its receptor, cyclophilin. This invention is further directed to a method for treating host versus graft disease following blood marrow transplantation by transfecting stem cells so that after introduction into a patient the stem cells will express the modified cyclophilin, and, as necessary, administer the modified cyclosporin A to the patient.

**French Abstract**

Cette invention se rapporte a une cyclosporine A modifiee et a une version modifiee par genie genetique de son recepteur, la cyclophiline. Cette invention se rapporte en outre a un procede pour traiter la reaction du greffon contre l'hote apres une greffe de moelle osseuse par transfection de cellules souches, pour que, apres introduction dans le corps d'un patient, les cellules souches expriment la cyclophiline modifiee et, si necessaire, administrent la cyclosporine A modifiee dans le corps du patient.

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DIALOG(R)File 349:PCT Fulltext

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00535811 \*\*Image available\*\*

**HISTONE DEACETYLASES, AND USES RELATED THERETO  
HISTONE&shy;DESACETYLASES ET LEURS UTILISATIONS**

**Patent Applicant/Assignee:**

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, PRESIDENT AND FELLOWS OF  
HARVARD COLLEGE, 17 Quincy Street, Cambridge, MA 02138, US

**Inventor(s):**

SCHREIBER Stuart L , SCHREIBER, Stuart, L., 434 Marlborough Street,  
Boston, MA 02115, US  
TAUNTON Jack, TAUNTON, Jack, 96 Willow Avenue &1, Somerville, MA 02144,  
US  
HASSIG Christian A, HASSIG, Christian, A., 68R Dane Street, Somerville,  
MA 02143, US  
JAMISON Timothy F, JAMISON, Timothy, F., 64 Frost Street &1, Cambridge,  
MA 02140, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9735990 A2 19971002  
Application: WO 97US5275 19970326 (PCT/WO US9705275)  
Priority Application: US 96624735 19960326

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW  
MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN GH KE LS  
MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE  
IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Main International Patent Class: C12N-015/55;

International Patent Class: C12N-009/16; C12N-015/63; A01K-067/027;  
A61K-048/00; C12Q-001/68; A61K-038/07; C07K-005/12; A61K-031/105;  
C07C-229/44; C07K-016/40; C12Q-001/44;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 51497

#### English Abstract

The present invention concerns proteins encoded by a family of genes,  
termed here < i> HDx< /i> &shy;related genes, which are involved in the  
control of chromatin structure and, thus in transcription and  
translation. The present invention makes available compositions and  
methods that can be utilized, for example, to control cell proliferation  
and differentiation < i> in vitro< /i> and < i> in vivo.< /i>

#### French Abstract

La presente invention concerne des proteines codees par une famille de  
genes, appeles, dans le present contexte, genes apparentes < i> HDx< /i>  
, qui sont impliquees dans la regulation de la structure de la chromatine  
et, par consequent dans les processus de transcription et de traduction.  
La presente invention decrit des compositions et des procedes  
utilisables, par exemple, pour reguler la proliferation et la  
differentiation cellulaires, tant < i> in vitro< /i> qu'< i> in vivo< /i>

1/5/9

DIALOG(R) File 349:PCT Fulltext

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00436395

#### LACTACYSTIN ANALOGS

#### ANALOGUES DE LACTACYSTINE

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE

SCHREIBER Stuart L

STANDAERT Robert F

FENTEANY Gabriel

JAMISON Timothy F

Inventor(s):

SCHREIBER Stuart L

STANDAERT Robert F

FENTEANY Gabriel

JAMISON Timothy F

Patent and Priority Information (Country, Number, Date):

Patent: WO 9632105 A1 19961017

Application: WO 96US5072 19960412 (PCT/WO US9605072)

Priority Application: US 95421583 19950412

Designated States: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB

GE HU IS JP KE KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO  
RU SD SE SG SI TM TR TT UA UG US UZ VN KE LS MW SD SZ UG AM AZ BY KG KZ  
MD RU TJ TM AT DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI  
CM GA GN ML MR TD TG

Main International Patent Class: A61K-031/395;

International Patent Class: A61K-038/00;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 39156

English Abstract

Compounds related to lactacystin and lactacystin 'beta'-lactone,  
pharmaceutical compositions containing the compounds, and methods of use.

Japanese Abstract

L'invention porte sur des composés liés à la lactacystine et à la  
lactacystine 'beta'-lactone, des compositions pharmaceutiques contenant  
ces composés et leurs procédés d'utilisation.

1/5/10

DIALOG(R) File 349:PCT Fulltext

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00418248

NEW METHOD FOR IDENTIFYING AND EVALUATING BIOLOGICALLY ACTIVE MOLECULES  
NOUVELLE METHODE D'IDENTIFICATION ET D'EXAMEN DE MOLECULES A ACTIVITE  
BIOLOGIQUE

Patent Applicant/Assignee:

ARIAD GENE THERAPEUTICS INC

SCHREIBER Stuart L

CRABTREE Gerald R

HOLT Dennis A

ZOLLER Mark J

Inventor(s):

SCHREIBER Stuart L

CRABTREE Gerald R

HOLT Dennis A

ZOLLER Mark J

Patent and Priority Information (Country, Number, Date):

Patent: WO 9613613 A1 19960509

Application: WO 95US14177 19951101 (PCT/WO US9514177)

Priority Application: US 94332995 19941101; US 95400800 19950307; US  
95480286 19950607

Designated States: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU  
IS JP KE KG KP LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG  
SI SK TJ TM TT US UZ VN KE LS MW SD SZ UG AT BE CH DE DK ES FR GB GR IE  
IT LU MC NL PT BJ CF CG CI CM GA GN ML MR NE SN TD TG

Main International Patent Class: C12Q-001/68;

International Patent Class: G01N-033/53;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 7748

English Abstract

This invention concerns materials, methods and applications relating to  
the multimerizing of protein mediators of biological events using  
synthetic, preferably non-peptidic, dimerizing agents or CIDs.

Japanese Abstract

L'invention porte sur des matériaux, des méthodes et des applications  
relatifs à la multimerisation des médiateurs protéiques d'événements  
biologiques à l'aide d'agents synthétiques de dimérisation de préférence  
non peptidiques, ou CID (inducteurs chimiques de dimérisation).

1/5/11  
DIALOG(R)File 349:PCT Fulltext  
(c) 2001 WIPO/MicroPat. All rts. reserv.

00374088    \*\*Image available\*\*  
**SEQUENCE-SPECIFIC GLYCOCONJUGATE TRANSCRIPTIONAL ANTAGONISTS**  
**GLYCOCONJUGES A SPECIFICITE DE SEQUENCE ANTAGONISTES DE TRANSCRIPTION**

Patent Applicant/Assignee:

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY  
YALE UNIVERSITY

THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE

Inventor(s):

HO Steffan N

SCHREIBER Stuart L

DANISHEFSKY Samuel J

CRABTREE Gerald R

Patent and Priority Information (Country, Number, Date):

Patent: WO 9505389 A1 19950223

Application: WO 94US9123 19940815 (PCT/WO US9409123)

Priority Application: US 93109271 19930818

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International Patent Class: C12Q-001/68;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20243

English Abstract

The invention provides glycoconjugates which bind polynucleotides in a sequence-selective manner and/or preferentially displace or inhibit binding of transcription factors to their recognition sites on DNA. The DNA-binding glycoconjugates of the invention, exemplified by calicheamicin-MG (as shown in the figure), are used as selective transcriptional antagonists, among other uses.

Japanese Abstract

L'invention porte sur des glycoconjuges qui fixent des polynucleotides par selection de sequence et/ou déplacent ou inhibent la fixation des facteurs de transcription a leurs sites de reconnaissance sur l'ADN. Lesdits glycoconjuges comme l'illustre la calicheamicine-MG (fig. 1) servent notamment d'antagonistes selectifs de transcriptions.

1/5/12

DIALOG(R)File 349:PCT Fulltext  
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00371292  
**REGULATED APOPTOSIS**  
**APOPTOSE REGULEE**

Patent Applicant/Assignee:

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY  
PRESIDENT AND FELLOWS OF HARVARD COLLEGE

Inventor(s):

CRABTREE Gerald R

SCHREIBER Stuart L

SPENCER David M

WANDLESS Thomas J

BELSHAW Peter

Patent and Priority Information (Country, Number, Date):

Patent: WO 9502684 A1 19950126

Application: WO 94US8008 19940718 (PCT/WO US9408008)

Priority Application: US 9393499 19930716; US 94179143 19940107; WO 94US1617 19940214

Designated States: AT AU BB BG BR BY CA CH CN CZ DE DK ES FI GB HU JP KP KR  
KZ LH LU LV MG NL NO NZ PL PT RO RU SD SE SK UA UZ VN AT BE CH DE DK ES



FR GB GR IE IT NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG  
Main International Patent Class: C12N-005/00;  
International Patent Class: C12N-015/00; C07H-015/12; C07K-015/00;  
A61K-031/70;  
Publication Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 34042

#### English Abstract

We have developed a general procedure for the regulated (inducible) dimerization or oligomerization of intracellular proteins and disclose methods and materials for using that procedure to regulatably initiate cell-specific apoptosis (programmed cell death) in genetically engineered cells.

#### Japanese Abstract

On a developpe un processus general de dimerisation ou d'oligomerisation regulee (pouvant etre induite) de proteines intracellulaires. Des procedes et des substances sont decrites, lesquels permettent d'amorcer de maniere regulable l'apoptose (mort cellulaire programme) dans des cellules produites par technique genetique.

1/5/13

DIALOG(R)File 349:PCT Fulltext  
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00357642 \*\*Image available\*\*

#### REGULATED TRANSCRIPTION OF TARGETED GENES AND OTHER BIOLOGICAL EVENTS TRANSCRIPTION REGULEE DE GENES CIBLES ET D'AUTRES EVENEMENTS BIOLOGIQUES

Patent Applicant/Assignee:

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY  
PRESIDENT AND FELLOWS OF HARVARD COLLEGE

Inventor(s):

CRABTREE Gerald R  
SCHREIBER Stuart L  
SPENCER David M  
WANDLESS Thomas J  
BELSHAW Peter

Patent and Priority Information (Country, Number, Date):

Patent: WO 9418317 A1 19940818  
Application: WO 94US1617 19940214 (PCT/WO US9401617)  
Priority Application: US 9317931 19930212; US 9392977 19930716; US  
94179748 19940107

Designated States: AT AU BB BG BR CA CH CN CZ DE DK ES FI GB HU JP KP KR LK  
LU MG MN MW NL RO RU SD SE SK AT BE CH DE DK ES FR GB GR IE IT LU MC NL  
PT SE BF BJ CF CM GA GN ML MR NE SN TD TG

Main International Patent Class: C12N-015/00;  
International Patent Class: C12N-005/00; C12N-005/06; C12N-015/11;  
C12P-021/06; C12P-021/00;  
Publication Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 32431

#### English Abstract

Dimerization and oligomerization of proteins are general biological control mechanisms that contribute to the activation of numerous cellular processes. We have developed a general procedure for the regulated (inducible) dimerization or oligomerization of intracellular proteins. As outlined in the figure, any two target proteins can be induced to associate by treating the cells or organisms that harbor them with cell permeable, synthetic ligands. In summary, we have induced: (1) the intracellular aggregation of the cytoplasmic tail of the zeta chain of the T cell receptor (TCR)-CD3 complex thereby leading to signaling and transcription of a reporter gene, (2) the homodimerization of the

cytoplasmic tail of the Fas receptor thereby leading to cell-specific apoptosis (programmed cell death) and (3) the heterodimerization of a DNA-binding domain (Gal4) and a transcription- activation domain (VP16) thereby leading to direct transcription of a reporter gene.

#### Japanese Abstract

La dimerisation et l'oligomerisation de proteines sont des mecanismes generaux de commandes biologiques contribuant a l'activation de nombreux processus cellulaires. Nous avons mis au point un procede general de dimerisation ou d'oligomerisation regulee (inductible) de proteines intracellulaires. Comme l'illustre la figure 15, on peut provoquer l'association de l'une ou l'autre de deux proteines cibles en traitant les cellules ou les organismes qui les contiennent avec des ligands synthetiques permeables aux cellules. En resume, nous avons provoque: (1) l'aggregation intracellulaire de la queue cytoplasmique de la chaine aux etats du complexe recepteur (TCR)-CD3 de cellules T, ce qui conduit a la signalisation et a la transcription d'un gene reporteur, (2) l'homodimerisation de la queue cytoplasmique du recepteur de Fas, ce qui implique une apoptose specifique a la cellule (mort cellulaire programmee) et (3) l'heterodimerisation d'un domaine de liaison de l'ADN (Gal4) et d'un domaine de transcription-activation (VP16), ce qui provoque la transcription directe d'un gene reporteur.

1/5/14

DIALOG(R)File 349:PCT Fulltext

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00316200

#### DETECTION OF IMMUNOSUPPRESSANTS

#### DETECTION D'IMMUNOSUPPRESSEURS

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY

Inventor(s):

SCHREIBER Stuart L

FRIEDMAN Jeffrey S

WEISSMAN Irving L

LIU Jun

Patent and Priority Information (Country, Number, Date):

Patent: WO 9303364 A1 19930218

Application: WO 92US6334 19920730 (PCT/WO US9206334)

Priority Application: US 91740175 19910805

Designated States: CA JP AT BE CH DE DK ES FR GB GR IT LU MC NL SE

Main International Patent Class: G01N-033/50;

International Patent Class: C12N-009/00; C12Q-001/00; A12K-035/12;

A12K-037/50; A12K-037/00;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 7864

#### English Abstract

A method of evaluating the immunosuppressive activity of a compound including contacting the compound with calcineurin and determining the ability of the compound to bind to the calcineurin. The ability to bind to the calcineurin is positively correlated to the immunosuppressive activity of the compound.

#### Japanese Abstract

Un procede d'evaluation de l'activite immunosuppressive d'un compose consiste a mettre en contact le compose avec de la calcineurine et a determiner la capacite du compose a se lier a la calcineurine. La capacite de fixation du compose sur la calcineurine est en correlation positive avec l'activite immunosuppressive du compose.

1/5/15

DIALOG(R) File 349:PCT Fulltext  
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00274491

**RECEPTOR FOR FK-506**

**RECEPTEUR POUR FK-506**

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE  
YALE UNIVERSITY

Inventor(s):

**SCHREIBER Stuart L**

**HARDING Matthew W**

Patent and Priority Information (Country, Number, Date):

Patent: WO 9104321 A1 19910404

Application: WO 90US5449 19900925 (PCT/WO US9005449)

Priority Application: US 89412088 19890925; US 90464978 19900116

Designated States: AT BE CH DE DK ES FR GB IT JP LU NL SE

Main International Patent Class: C12N-009/00;

International Patent Class: C12N-009/90;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 2241

#### English Abstract

Binding proteins of both human and bovine origin for the immunosuppressant FK506 have isomerase activity which is inhibited by FK506 but not by cyclosporin a. The N-terminal sequence of both proteins is H2-N-Gly-Val-Gln-Val-Glu-Thr-Ile-Ser-Pro-Gly-Asp-Gly-Arg-Thr-Phe-Pro-Lys-Arg- Gly-Gln-Thr-Cys-Val-Val-His-Tyr-Thr-Gly-Met-Leu-Glu-Asp-Gly-Lys-Lys-Phe-Asp-S er-Ser-Arg. The entire sequence of the human protein deduced from the cloned human gene contains in addition: Asp-Arg-Asn-Lys-Pro-Phe-Lys-Phe-Met-Leu-Gly-Lys-Gln-Glu-Val-Ile-Arg-Gly-Trp-G lu-Glu-Gly-Val-Ala-Gln-Met-Ser-Val-Gly-Gln-Arg-Ala-Lys-Leu-Thr-Ile-Ser-Pro-As p-Tyr-Ala-Tyr-Gly-Ala-Thr-Gly-His-Pro-Gly-Ile-Ile-Pro-Pro-His-Ala-Thr-Leu-Val -Phe-Asp-Val-Glu-Leu-Leu-Lys-Leu-Glu.

#### Japanese Abstract

Des proteines de liaison tant d'origine humaine que bovine pour l'immunosuppresseur FK506 ont une activite d'isomerase qui est inhibee par FK506 mais non par la cyclosporine A. La sequence a terminaison N des deux proteines est H2-N-Gly-Val-Gln-Val-Glu-Thr-Ile-Ser-Pro-Gly-Asp-Gly-Arg-Thr-Phe-Pro-Lys-Arg- Gly-Gln-Thr-Cys-Val-Val-His-Tyr-Thr-Gly-Met-Leu-Glu-Asp-Gly-Lys-Lys-Phe-Asp-S er-Ser-Arg. La sequence entiere de la proteine humaine deduite du gene humain clone contient de plus: Asp-Arg-Asn-Lys-Pro-Phe-Lys-Phe-Met-Leu-Gly-Lys-Gln-Glu-Val-Ile-Arg-Gly-Trp-G lu-Glu-Gly-Val-Ala-Gln-Met-Ser-Val-Gly-Gln-Arg-Ala-Lys-Leu-Thr-Ile-Ser-Pro-As p-Tyr-Ala-Tyr-Gly-Ala-Thr-Gly-His-Pro-gly-Ile-Ile-Pro-Pro-His-Ala-Thr-Leu-Val -Phe-Asp-Val-Glu-Leu-leu-Lys-Leu-Glu.

1/5/16

DIALOG(R) File 349:PCT Fulltext

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00215369

**PROCESS FOR PREPARING SYNTHETIC PERIPLANONE-B**

**PROCEDE DE PREPARATION DE PERIPLANONE-B SYNTHETIQUE**

Patent Applicant/Assignee:

YALE UNIVERSITY

Inventor(s):

**SCHREIBER Stuart L**

Patent and Priority Information (Country, Number, Date):

Patent: WO 8600308 A1 19860116

Application: WO 85US1109 19850613 (PCT/WO US8501109)

Priority Application: US 84621574 19840618

Designated States: AT BE CH DE FR GB IT JP LU NL SE

Main International Patent Class: C07D-493/10;

International Patent Class: C07D-303/32; C07C-049/647; C07C-049/623;  
C07C-035/31; C07C-037/20; C07D-303/34; C07C-149/32;

Publication Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 5550

English Abstract

An improved process for synthesizing periplanone-B, the sex attractant and sex excitant pheromone of the American cockroach, *Periplaneta americana*.

Japanese Abstract

On decrit un procede ameliore de synthese de periplanone-B, la pheromone d'attraction et d'excitation sexuelle produite par la blatte americaine, *Periplaneta americana*.

?e au=zhu heng

Ref	Items	Index-term
E1	1	AU=ZHU HELEN
E2	1	AU=ZHU HELEN H
E3	0	*AU=ZHU HENG
E4	4	AU=ZHU HENGYI
E5	1	AU=ZHU HONGWU
E6	2	AU=ZHU HUA
E7	4	AU=ZHU HUI
E8	2	AU=ZHU JI
E9	1	AU=ZHU JIABI J
E10	2	AU=ZHU JIAN-GANG
E11	1	AU=ZHU JIANHUI
E12	2	AU=ZHU JIANZHONG

Enter P or PAGE for more

?e au=snyder michale

Ref	Items	Index-term
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E2	1	AU=SNYDER MICHAEL THOMS
E3	0	*AU=SNYDER MICHAEL
E4	2	AU=SNYDER NANCY J
E5	1	AU=SNYDER NANCY JUNE
E6	2	AU=SNYDER PAUL J JR
E7	1	AU=SNYDER PETER J
E8	1	AU=SNYDER PHILIP H
E9	2	AU=SNYDER RANDALL A
E10	1	AU=SNYDER RANDY B
E11	7	AU=SNYDER RICHARD
E12	2	AU=SNYDER RICHARD O

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?s au=snyder michael?

S2 14 AU=SNYDER MICHAEL?

?s s2 and microarray

14 S2

785 MICROARRAY

S3 0 S2 AND MICROARRAY

?s s2 and array

14 S2

50084 ARRAY

S4 1 S2 AND ARRAY

?t 4/5/1

4/5/1

DIALOG(R)File 349:PCT Fulltext

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00654500 \*\*Image available\*\*

A METHOD AND APPARATUS FOR MAKING AN ELASTOMERIC LAMINATE WEB  
PROCEDE ET APPAREIL POUR LA FABRICATION DE VOILES ELASTOMERES LAMINES

Patent Applicant/Assignee:

KIMBERLY-CLARK WORLDWIDE INC, KIMBERLY-CLARK WORLDWIDE, INC. , 401 North Lake Street, Neenah, WI 54956 , US

Inventor(s):

HEATH Mark Gordon, HEATH, Mark, Gordon , 1067 Rock Ledge Lane, Neenah, WI 54956 , US

CAHALL James Louis, CAHALL, James, Louis , N241 Woodstock Lane, Appleton, WI 54915 , US

FRENCH Timothy Alan, FRENCH, Timothy, Alan , 19 Weantinock Drive, New Milford, CT 06776 , US

JOHNSON Eric Donald, JOHNSON, Eric, Donald , 5255 Grandview Road, Larsen, WI 54947 , US

SNYDER Michael A , SNYDER, Michael, A. , N1559 County T, Hortonville, WI 54944 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9937480 A1 19990729

Application: WO 99US1064 19990119 (PCT/WO US9901064)

Priority Application: US 9810456 19980121

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV

MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: B32B-031/00;

International Patent Class: B65H-018/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 9988

English Abstract

The present invention provides a process for making an elastomeric web. Generally stated, the process includes moving a first web (26) along an appointed machine-direction (28) of the process, and delivering a plurality of elastomeric strands (30) moving along the machine-direction. The elastomeric strands (30) have been elastomerically stretched to an elongation of at least about 50 percent, and the plurality of elastomeric strands (30) have been laterally distributed along an appointed cross-direction (32) of the process. The elastomeric strands (30) are positioned at a spaced distance (34) from the first web (26), and an adhesive is applied to the elastomeric strands (30) and the first web (26) while the elastomeric strands are at the spaced distance (34) from the first web (26). A second web (36) is laminated to the first web (26) with the elastomeric strands (30) sandwiched between the first and second webs to provide an elastomeric laminate, composite web (24) attached together with the adhesive. The composite web (24) is allowed to elastomerically contract lengthwise by at least about 3 percent, and the composite web (24) undergoes a traverse winding into a roll.

French Abstract

La presente invention concerne un procede de fabrication de voiles elastomeres. De facon generale, ce procede consiste C amener un premier voile (26) dans le sens de progression (28) de l'installation de fabrication, et C presenter une pluralite de fils elastomeres (30) qui se deplacent dans le meme sens. Les fils elastomeres (30) ont ete etires C au moins 50 % environ de leur longueur initiale et la pluralite de ces fils (30) a ete repartie transversalement par rapport au sens de progression du processus. Les fils elastomeres (30) se trouvant C l'ecart et C une distance determinee (34) du premier voile (26), un produit adhesif est applique sur lesdits fils (30) et ledit voile (26). Un second voile (36) est lamine sur le premier voile (26), les fils (30) se trouvant intercales entre le premier et le second voile. Le voile elastomere lamine composite (24) ainsi obtenu est fixe au moyen d'un adhesif. Apres retraction longitudinale d'au moins 3 %, le voile composite (24) est enroule par bobinage C spires croisees.

Set	Items	Description
S1	16	AU=SCHREIBER STUART L
S2	14	AU=SNYDER MICHAEL?
S3	0	S2 AND MICROARRAY
S4	1	S2 AND ARRAY
?s s2 and (peptid? or protein?)		
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	49927	PEPTID?
	73629	PROTEIN?
S5	5	S2 AND (PEPTID? OR PROTEIN?)
?s s5 and (sample or specimen)		
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	97544	SAMPLE
	10582	SPECIMEN
S6	4	S5 AND (SAMPLE OR SPECIMEN)
?t 4/6/1		

4/6/1  
00654500 \*\*Image available\*\*  
A METHOD AND APPARATUS FOR MAKING AN ELASTOMERIC LAMINATE WEB  
PROCEDE ET APPAREIL POUR LA FABRICATION DE VOILES ELASTOMERES LAMINES  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 9988  
Publication Year: 1999  
?  
?t 6/6/1

6/6/1  
00785638  
HAIR CONDITIONING COMPOSITION COMPRISING CARBOXYLIC ACID/CARBOXYLATE  
COPOLYMER AND MOISTURIZING AGENT  
COMPOSITION CAPILLAIRE TONIFIANTE COMPRENANT UN ACIDE  
CARBOXYLIQUE/COPOLYMER CARBOXYLATE ET UN AGENT HYDRATANT  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 13396  
Publication Year: 2001  
?t 6/6/2-4

6/6/2  
00444990 \*\*Image available\*\*  
NOVEL Numa-INTERACTING PROTEINS AND METHODS FOR THEIR IDENTIFICATION  
PROTEINES A INTERACTION Numa ET PROCEDES D'IDENTIFICATION CORRESPONDANTS  
Publication Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 30771  
Publication Year: 1996

6/6/3  
00285146  
HAIR STYLING CONDITIONERS  
DEMELANTS DE MISE EN FORME DES CHEVEUX  
Publication Language: English  
Fulltext Availability:  
Detailed Description  
Claims

Fulltext Word Count: 11571  
Publication Year: 1991

6/6/4  
00285145  
HAIR STYLING SHAMPOOS  
SHAMPOOINGS DE MISE EN FORME DES CHEVEUX  
Publication Language: English  
Fulltext Availability:  
Detailed Description  
Claims

Fulltext Word Count: 11777  
Publication Year: 1991  
?logoff hold

02may01 16:36:51 User026066 Session D6427.2  
Sub account: 3776-010140 LAUNCHCYTE BEJ  
\$7.32 1.541 DialUnits File349  
\$86.70 17 Type(s) in Format 5  
\$0.00 5 Type(s) in Format 6  
\$86.70 22 Types  
\$94.02 Estimated cost File349  
\$2.40 TELNET  
\$96.42 Estimated cost this search  
\$96.70 Estimated total session cost 1.600 DialUnits

### Status: Signed Off. (12 minutes)

### Status: Path 1 of [Dialog]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID dialog.com)  
Trying 3106...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 00.12.12D

Reconnected in file 349 02may01 16:49:40

NOTICE set ON to \$20.00

You will be prompted to confirm each TYPE or PRINT request where  
format charges exceeds \$20.00

Please enter SUBACCOUNT name/number:

?s (protein?/cl or peptid?/cl) and (array/cl or microarray/cl or chip/cl)

Is S (PROTEIN?/CL OR PEPTID?/CL) AN the SUBACCOUNT you want to use? (Y/N)

?n

Do you want to specify another SUBACCOUNT? (Y/N)

?y

Please enter SUBACCOUNT name/number:

?3776-010140 launchcyte bej

Is 3776-010140 LAUNCHCYTE BEJ the SUBACCOUNT you want to use? (Y/N)

?y

Subaccount is set to 3776-010140 LAUNCHCYTE BEJ

\* \* \*

File 349:PCT Fulltext 1983-2001/UB=20010419, UT=20010405  
(c) 2001 WIPO/MicroPat

Set Items Description

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>>>Term "CL" is not defined in file 349 and is ignored

>>>File 349 processing for PROTEIN? stopped at PROTEINSPEZIFISCHE

>>>File 349 processing for PEPTID? stopped at PEPTIDYLANTEIL

73629 PROTEIN?/CL

49927 PEPTID?/CL

50084 ARRAY/CL

916 MICROARRAY?/CL

37503 CHIP?/CL

S7 12456 (PROTEIN?/CL OR PEPTID?/CL) AND (ARRAY/CL OR  
MICROARRAY?/CL OR CHIP?/CL)

?e cl=peptide

Ref	Items	Index-term
E1	63800	AY=2000
E2	4	AY=2001
E3	0	*CL=PEPTIDE
E4	2	CN=AD
E5	6	CN=AE
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E7	1	CN=AI
E8	3	CN=AL
E9	8	CN=AM
E10	99	CN=AN
E11	85	CN=AR
E12	1061	CN=AT

Enter P or PAGE for more

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02may01 16:51:37 User026066 Session D6427.3

Sub account: 3776-010140 LAUNCHCYTE BEJ

\$2.18 0.459 DialUnits File349

\$2.18 Estimated cost File349

\$0.40 TELNET

\$2.58 Estimated cost this search

\$2.58 Estimated total session cost 0.459 DialUnits

### Status: Signed Off. (2 minutes)



### Status: Path 1 of [Dialog]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID dialog.com)  
Trying 3106...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\* HHHHHHHH SSSSSSSS?

### Status: Signing onto Dialog

\*\*\*\*\*

ENTER PASSWORD:

\*\*\*\*\* HHHHHHHH SSSSSSSS? \*\*\*\*\*

Welcome to DIALOG

### Status: Connected

Dialog level 00.12.12D

Last logoff: 02may01 16:51:37

Logon file001 02may01 17:53:28

NOTICE set ON to \$20.00

You will be prompted to confirm each TYPE or PRINT request where  
format charges exceeds \$20.00

Please enter SUBACCOUNT name/number:

?3776-010140 launchcyte bej

Is 3776-010140 LAUNCHCYTE BEJ the SUBACCOUNT you want to use? (Y/N)

?y

Subaccount is set to 3776-010140 LAUNCHCYTE BEJ

\* \* \*

File 1:ERIC 1966-2001/Apr 17

(c) format only 2001 The Dialog Corporation

Set Items Description

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Terminal set to DLINK

?b 349

02may01 17:53:53 User026066 Session D6428.1

Sub account: 3776-010140 LAUNCHCYTE BEJ

\$0.19 0.056 DialUnits File1

\$0.19 Estimated cost File1

\$0.08 TELNET

\$0.27 Estimated cost this search

\$0.27 Estimated total session cost 0.056 DialUnits

File 349:PCT Fulltext 1983-2001/UB=20010419, UT=20010405

(c) 2001 WIPO/MicroPat

Set Items Description

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29305 PROTEIN? ?/CM

21855 PEPTIDE? ?/CM

16459 ARRAY? ?/CM

167 MICROARRAY? ?/CM

S1 1134 (PROTEIN? ?/CM OR PEPTIDE? ?/CM) AND (ARRAY? ?/CM OR  
MICROARRAY? ?/CM)

?s s1 and polyethylene()glycol

1134 S1

65081 POLYETHYLENE

56798 GLYCOL

27239 POLYETHYLENE(W)GLYCOL

S2 401 S1 AND POLYETHYLENE()GLYCOL

?s (protein? ?/cm or peptide? ?/cm) (n10) microarray? ?/cm (n10) polyethylene()glycol

29305 PROTEIN? ?/CM

21855 PEPTIDE? ?/CM

167 MICROARRAY? ?/CM

65081 POLYETHYLENE  
56798 GLYCOL  
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?s (protein? ?/cm or peptide? ?/cm) (n20) microarray? ?/cm  
29305 PROTEIN? ?/CM  
21855 PEPTIDE? ?/CM  
167 MICROARRAY? ?/CM  
S4 19 (PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) MICROARRAY? ?/CM  
?t 4/5/1

4/5/1

DIALOG(R) File 349: PCT Fulltext

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00791355

**SECRETORY MOLECULES**

**MOLECULES SECRETOIRES**

Patent Applicant/Assignee:

INCYTE GENOMICS INC, 3160 Porter Drive, Palo Alto, CA 94304, US, US  
(Residence), US (Nationality), (For all designated states except: US)  
Patent Applicant/Inventor:  
HODGSON David M, 567 Addison Avenue, Palo Alto, CA 94304, US, US  
(Residence), US (Nationality), (Designated only for: US)  
LINCOLN Stephen E, 725 Sapphire Street, Redwood City, CA 94061, US, US  
(Residence), US (Nationality), (Designated only for: US)  
RUSSO Frank D, 1583 Courdillaeras Road, Redwood City, CA 94062, US, US  
(Residence), US (Nationality), (Designated only for: US)  
SPIRO Peter A, 3875 Park Boulevard, Apt. B16, Palo Alto, CA 94306, US, US  
(Residence), US (Nationality), (Designated only for: US)  
BANVILLE Steven C, 604 San Diego Avenue, Sunnyvale, CA 94086, US, US  
(Residence), US (Nationality), (Designated only for: US)  
BRATCHER Shawn R, 550 Ortega Avenue #B321, Mountain View, CA 94040, US,  
US (Residence), US (Nationality), (Designated only for: US)  
DUFOUR Gerard E, 5327 Greenridge Road, Castro Valley, CA 94552-2619, US,  
US (Residence), US (Nationality), (Designated only for: US)  
COHEN Howard J, 3272 Cowper Street, Palo Alto, CA 94306-3004, US, US  
(Residence), US (Nationality), (Designated only for: US)  
ROSEN Bruce H, 177 Hanna Way, Menlo Park, CA 94025, US, US (Residence),  
US (Nationality), (Designated only for: US)  
SHAH Purvi, 859 Salt Lake Drive, San Jose, CA 95133, US, US (Residence),  
IN (Nationality), (Designated only for: US)  
CHALUP Michael S, 183 Acalanes Drive, Apt. 6, Sunnyvale, CA 94086, US, US  
(Residence), US (Nationality), (Designated only for: US)  
HILLMAN Jennifer L, 230 Monroe Drive, #17, Mountain View, CA 94040, US,  
US (Residence), US (Nationality), (Designated only for: US)  
JONES Anissa Lee, 445 South 15th Street, San Jose, CA 95112, US, US  
(Residence), US (Nationality), (Designated only for: US)  
YU Jimmy Y, 37330 Portico Terrace, Fremont, CA 94536-7901, US, US  
(Residence), US (Nationality), (Designated only for: US)  
GREENAWALT Lila B, 1596 Ballantree Way, San Jose, CA 95118-2106, US, US  
(Residence), US (Nationality), (Designated only for: US)  
PANZER Scott R, 965 East El Camino, #621, Sunnyvale, CA 94087, US, US  
(Residence), US (Nationality), (Designated only for: US)  
ROSEBERRY Ann M, 725 Sapphire Street, Redwood City, CA 94061, US, US  
(Residence), US (Nationality), (Designated only for: US)  
WRIGHT Rachel J, 339 Anna Way, Mountain View, CA 94043, US, US  
(Residence), NZ (Nationality), (Designated only for: US)  
CHEN Wensheng, 210 Easy Street, #25, Mountain View, CA 94043, US, US  
(Residence), CN (Nationality), (Designated only for: US)  
LIU Tommy F, 201 Ottilia Street, Daly City, CA 94014, US, US (Residence),  
US (Nationality), (Designated only for: US)  
YAP Pierre E, 201 Happy Hollow Court, Lafayette, CA 94549-6243, US, US  
(Residence), US (Nationality), (Designated only for: US)  
STOCKDREHER Theresa K, 1596 Ontario Drive, #2, Sunnyvale, CA 94087, US,  
US (Residence), US (Nationality), (Designated only for: US)  
AMSHEY Stefan, 1541 Canna Court, Mountain View, CA 94043, US, US  
(Residence), US (Nationality), (Designated only for: US)  
FONG Willy T, 573 Cambridge Street, San Francisco, CA 94134, US, US

(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

HAMLET-COX Diana (et al) (agent), Incyte Genomics, Inc., 3160 Porter Drive, Palo Alto, CA 94304, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200123558 A2 20010405 (WO 0123558)

Application: WO 2000US25610 20000919 (PCT/WO US0025610)

Priority Application: US 99156624 19990928; US 99156625 19990928; US

99168611 19991202; US 99168613 19991202; US 99168614 19991202

Parent Application/Grant:

Related by Continuation to: US 99156624 19990928 (CIP); US 99156625

19990928 (CIP); US 99168614 19991202 (CIP); US 99168611 19991202 (CIP);

US 99168613 19991202 (CIP)

Designated States: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ

DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ

LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG

SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12N-015/12

International Patent Class: C07K-014/705; C12Q-001/68; G01N-033/50;

A01K-067/027

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 65628

English Abstract

The present invention provides purified secretory polynucleotides (sptm). Also encompassed are the polypeptides (SPTM) encoded by sptm. The invention also provides for the use of sptm, or complements, oligonucleotides, or fragments thereof in diagnostic assays. The invention further provides for vectors and host cells containing sptm for the expression of SPTM. The invention additionally provides for the use of isolated and purified SPTM to induce antibodies and to screen libraries of compounds and the use of anti-SPTM antibodies in diagnostic assays. Also provided are microarrays containing sptm and methods of use.

French Abstract

L'invention concerne des polynucleotides secretoires purifies (SPTM), ainsi que des polypeptides (SPTM) codes par les SPTM. L'invention concerne egalement l'utilisation de SPTM, ou de complements, ou d'oligonucleotides, ou de fragments de ces derniers dans des jeux ordonnes d'echantillons de diagnostic. L'invention concerne en outre des vecteurs et des cellules hotes contenant des SPTM pour l'expression de SPTM. L'invention concerne aussi l'utilisation de SPTM isolees et purifies pour induire des anticorps et pour examiner des bibliotheques de composees, et l'utilisation d'anticorps SPTM dans des jeux ordonnes d'echantillons de diagnostic. L'invention concerne enfin des jeux ordonnes de microechantillons contenant des SPTM et des procedes d'utilisation.

Legal Status (Type, Date, Text)

Publication 20010405 A2 Without international search report and to be republished upon receipt of that report.

?ds

Set	Items	Description
S1	1134	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) AND (ARRAY? ?/CM OR MICRO-ARRAY? ?/CM)
S2	401	S1 AND POLYETHYLENE() GLYCOL
S3	0	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N10) MICROARRAY? ?/CM (N-10) POLYETHYLENE() GLYCOL
S4	19	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) MICROARRAY? ?/CM

?s s2 and 'aerosol? or mist? or nebuli?'

401 S2  
 18176 AEROSOL?  
 9322 MIST?  
 4075 NEBULI?  
 S5 73 S2 AND (AEROSOL? OR MIST? OR NEBULI?)  
 ?s s5 and biolog?  
 73 S5  
 72708 BIOLOG?  
 S6 67 S5 AND BIOLOG?  
 ?s (protein? ?/cm or peptide? ?/cm) (n20) (dot? ?/cm or spot? ?/cm or microdot? ?/cm)  
 29305 PROTEIN? ?/CM  
 21855 PEPTIDE? ?/CM  
 2871 DOT? ?/CM  
 3670 SPOT? ?/CM  
 11 MICRODOT? ?/CM  
 S7 229 (PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) (DOT? ?/CM OR  
 SPOT? ?/CM OR MICRODOT? ?/CM)  
 ?s s7 and (glycerol or polyethylene()glycol)  
 229 S7  
 33632 GLYCEROL  
 65081 POLYETHYLENE  
 56798 GLYCOL  
 27239 POLYETHYLENE(W) GLYCOL  
 S8 133 S7 AND (GLYCEROL OR POLYETHYLENE() GLYCOL)  
 ?s s8 and (array or microarray)  
 133 S8  
 50084 ARRAY  
 785 MICROARRAY  
 S9 46 S8 AND (ARRAY OR MICROARRAY)  
 ?s s9 and chip  
 46 S9  
 27617 CHIP  
 S10 21 S9 AND CHIP  
 ?s s10 and scan?  
 21 S10  
 55291 SCAN?  
 S11 20 S10 AND SCAN?  
 ?s s11 and (fluor? or chrom?)  
 >>>File 349 processing for FLUOR? stopped at FLUORCOPOLYMERE  
 >>>File 349 processing for CHROM? stopped at CHROMATOGRAPHIPD  
 20 S11  
 16161 FLUOR?  
 44775 CHROM?  
 S12 20 S11 AND (FLUOR? OR CHROM?)  
 ?s s12 and (mist? or aerosol? or nebuli?)  
 20 S12  
 9322 MIST?  
 18176 AEROSOL?  
 4075 NEBULI?  
 S13 2 S12 AND (MIST? OR AEROSOL? OR NEBULI?)  
 ?t 13/5/1

13/5/1  
 DIALOG(R)File 349:PCT Fulltext  
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00741507

**REPLICA AMPLIFICATION OF NUCLEIC ACID ARRAYS**  
**AMPLIFICATION PAR REPLIQUE DE RESEAUX D'ACIDES NUCLEIQUES**

Patent Applicant/Assignee:

PRESIDENT AND FELLOWS OF HARVARD COLLEGE, Harvard College, 17 Quincy  
 Street, Cambridge, MA 02138, US, US (Residence), US (Nationality)

Inventor(s):

CHURCH George M, 218 Kent Street, Brookline, MA 02146, US  
 MITRA Robi D, 21 Hammond Pond Parkway, #3, Chestnut Hill, MA 02147, US  
 Legal Representative:

IWANICKI John P, Banner & Witcoff, Ltd., 28 State Street, 28th Floor,  
 Boston, MA 02109, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200053812 A2 20000914 (WO 0053812)  
Application: WO 2000US6390 20000310 (PCT/WO US0006390)  
Priority Application: US 99267496 19990312  
Designated States: AU CA JP  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
Main International Patent Class: C12Q-001/68  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 39326

#### English Abstract

Disclosed are improved methods of making and using immobilized arrays of nucleic acids, particularly methods for producing replicas of such arrays. Included are methods for producing high density arrays of nucleic acids and replicas of such arrays, as well as methods for preserving the resolution of arrays through rounds of replication. Also included are methods which take advantage of the availability of replicas of arrays for increased sensitivity in detection of sequences on arrays. Improved methods of sequencing nucleic acids immobilized on arrays utilizing single copies of arrays and methods taking further advantage of the availability of replicas of arrays are disclosed. The improvements lead to higher fidelity and longer read lengths of sequences immobilized on arrays. Methods are also disclosed which improve the efficiency of multiplex PCR using arrays of immobilized nucleic acids.

#### French Abstract

L'invention concerne des methodes ameliorees permettant de fabriquer et d'utiliser des reseaux immobilises d'acides nucleiques, notamment des methodes permettant de produire des repliques de ces reseaux. L'invention concerne egalement des methodes permettant de produire des reseaux d'acides nucleiques a haute densite et repliques de ces reseaux ainsi que des methodes permettant de conserver la resolution des reseaux apres plusieurs replications. L'invention concerne egalement des methodes qui profitent de la disponibilite des repliques de reseaux pour augmenter la sensibilite pour la detection de sequences sur les reseaux. L'invention concerne en outre des methodes ameliorees permettant de sequencer les acides nucleiques immobilises sur des reseaux au moyen de copies uniques de reseaux ainsi que des methodes qui profitent egalement de la disponibilite des repliques de reseaux. Ces ameliorations permettent d'obtenir une fidelite plus elevee et des longueurs superieures de lectures de sequences immobilisees sur des reseaux. L'invention concerne enfin des methodes permettant d'ameliorer l'efficacite de la PCR multiplex au moyen de reseaux d'acides nucleiques immobilises.

#### Legal Status (Type, Date, Text)

Publication 20000914 A2 Without international search report and to be republished upon receipt of that report.  
Examination 20010315 Request for preliminary examination prior to end of 19th month from priority date  
?t 13/5/2

13/5/2

DIALOG(R) File 349:PCT Fulltext  
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00628739

#### ASSAYS FOR DETECTING MODULATORS OF CYTOSKELETAL FUNCTION METHODES DE DETECTION DE MODULATEURS DE FONCTION CYTOSQUELETTIQUE

Patent Applicant/Assignee:

BOARD OF TRUSTEES OF LELAND STANFORD JR UNIVERSITY, BOARD OF TRUSTEES OF LELAND STANFORD JR. UNIVERSITY, Office of Technology Licensing, Suite 350, 900 Welch Road, Palo Alto, CA 94304- 1850, US  
REGENTS OF THE UNIVERSITY OF CALIFORNIA, REGENTS OF THE UNIVERSITY OF CALIFORNIA, Office of Technology Transfer, Dept. 0910, 9500 Gilman Drive, La Jolla, CA 92093-0910, US  
Inventor's:

VALE Ron, VALE, Ron , 1439 - 15th Avenue, San Francisco, CA 94122 , US  
PIERCE Daniel, PIERCE, Daniel , 1723 - 9th Avenue, San Francisco, CA  
94122 , US  
SPUDICH James, SPUDICH, James , 3035 Country Club Street, Palo Alto, CA  
94304 , US  
GOLDSTEIN Lawrence S B, GOLDSTEIN, Lawrence, S., B. , 13384 Keegan Place,  
San Diego, CA 92130 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9911814 A1 19990311  
Application: WO 98US18368 19980903 (PCT/WO US9818368)  
Priority Application: US 9757895 19970904

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12Q-001/00;

International Patent Class: G01N-031/00; G01N-033/53; G01N-033/542;  
G01N-033/543; G01N-033/544; G01N-033/545; G01N-033/567;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 21436

English Abstract

Described herein are methods of identifying compounds which modulate the activity of the cytoskeletal system. The methods are rapid, convenient and sensitive. Preferably, the method is used to identify lead compounds that can be used as therapeutics, diagnostics and agricultural agents. Generally, test compounds are added to two cytoskeletal components which bind to one another, to determine whether the binding is affected by the test compound. Wherein the binding is affected, a compound which modulates the cytoskeletal system is identified.

French Abstract

L'invention se rapporte C des methodes d'identification de composes qui modulent l'activite du systeme cytosquelettique. Ces methodes sont rapides, efficaces et sensibles. De preference, ces methodes visent C identifier des composes de plomb susceptibles d'etre utilises en tant qu'agents therapeutiques, diagnostiques et agents destines C l'agriculture. Generalement, on ajoute les composes d'essai C deux composants qui se lient l'un C l'autre, de facon C evaluer l'influence du compose d'essai sur la liaison. Lorsque cette liaison est influencee par le compose d'essai, on en deduit que ce compose est un compose modulateur du systeme cytosquelettique.

?s (protein? ?/cm or peptid? ?/cm) and (microarray? ?/ab or microarray? ?/cm)

29305 PROTEIN? ?/CM

20606 PEPTID? ?/CM

48 MICROARRAY? ?/AB

167 MICROARRAY? ?/CM

S14 120 (PROTEIN? ?/CM OR PEPTID? ?/CM) AND (MICROARRAY? ?/AB OR MICROARRAY? ?/CM)

?s s14 and (5()micron?) or (five()micron)

120 S14

486743 5

44154 MICRON?

8482 5(W)MICRON?

89846 FIVE

22721 MICRON

185 FIVE(W)MICRON

S15 190 S14 AND (5()MICRON?) OR (FIVE()MICRON)

?s s14 and (5()micron? or five()micron?)

120 S14

486743 5

44154 MICRON?

8482 5(W)MICRON?

89846 FIVE  
44154 MICRON?  
516 FIVE(W)MICRON?  
S16 5 S14 AND (5())MICRON? OR FIVE()MICRON?)

?t 16/5/1

16/5/1  
DIALOG(R)File 349:PCT Fulltext  
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00782572 \*\*Image available\*\*  
METHOD AND DEVICE FOR PERFORMING OPERATIONS AT CHARGED MICROLOCATIONS  
PROCEDE ET DISPOSITIF PERMETTANT DE REALISER DES OPERATIONS SUR DES  
MICROSITES CHARGES

Patent Applicant/Assignee:

PICOGRAM INC, 2257 Old Middlefield Way, Mountain View, CA 94043, US, US  
(Residence), US (Nationality)

Inventor(s):

HERRICK Steven S, 12760 Dianne Drive, Los Altos, CA 94022, US,

Legal Representative:

HOLLAND Charles D (et al) (agent), Morrison & Foerster LLP, 755 Page Mill  
Road, Palo Alto, CA 94304-1018, US,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200115800 A1 20010308 (WO 0115800)

Application: WO 2000US23289 20000825 (PCT/WO US0023289)

Priority Application: US 99151158 19990827; US 2000174969 20000106

Designated States: JP

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: B01J-019/00

International Patent Class: C07H-021/00; G01N-033/543; C07K-001/04

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 16264

English Abstract

Methods and devices are provided for producing dense arrays of chemical entities. A substrate comprises a plurality of microlocations having microelectrodes connected to a network for connection to a computer to control the voltage and polarity at each of said microelectrodes. Means for producing electrically charged microparticles comprising at least one chemical moiety produce a mist of the particles which is directed to the surface of said substrate, where the microparticles are captured by microlocations of lower potential. By providing chemical moieties concurrently or sequentially, oligomers may be formed or small organic compounds synthesized. The resulting arrays may be used for screening samples for specific binding entities.

French Abstract

L'invention concerne des procedes et des dispositifs permettant de produire des matrices denses d'entites chimiques. Un substrat comprend plusieurs microsites presentant des microelectrodes connectees a un reseau afin de se connecter a un ordinateur permettant de reguler la tension et la polarite de chacune de ces microelectrodes. Des moyens de production de microparticules electriquement chargees comprennent au moins une fraction chimique produisant une brume de particules dirigees vers la surface dudit substrat, les microparticules y etant capturees par les microsites de potentiel inferieur. En fournissant des fractions chimiques conjointement ou sequentiellement, des oligomeres peuvent se former ou de petits composés organiques etre synthetises. Les matrices qui en resultent peuvent etre utilisees dans le criblage d'echantillons pour la fixation d'entites specifiques.

Legal Status (Type, Date, Text)

Publication 20010308 A1 With international search report.

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Set	Items	Description
S1	1134	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) AND (ARRAY? ?/CM OR MICRO-ARRAY? ?/CM)
S2	401	S1 AND POLYETHYLENE()GLYCOL
S3	0	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N10) MICROARRAY? ?/CM (N-10) POLYETHYLENE()GLYCOL
S4	19	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) MICROARRAY? ?/CM
S5	73	S2 AND (AEROSOL? OR MIST? OR NEBULI?)
S6	67	S5 AND BIOLOG?
S7	229	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) (DOT? ?/CM OR SPOT? ?/CM OR MICRODOT? ?/CM)
S8	133	S7 AND (GLYCEROL OR POLYETHYLENE()GLYCOL)
S9	46	S8 AND (ARRAY OR MICROARRAY)
S10	21	S9 AND CHIP
S11	20	S10 AND SCAN?
S12	20	S11 AND (FLUOR? OR CHROM?)
S13	2	S12 AND (MIST? OR AEROSOL? OR NEBULI?)
S14	120	(PROTEIN? ?/CM OR PEPTID? ?/CM) AND (MICROARRAY? ?/AB OR M-ICROARRAY? ?/CM)
S15	190	S14 AND (5()MICRON?) OR (FIVE()MICRON)
S16	5	S14 AND (5()MICRON? OR FIVE()MICRON?)

?t 16/5/2-5

Estimated cost of output requested is: \$20.40  
Are you ready to receive all output? (Yes/No/Help)  
?y

16/5/2

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00746978 \*\*Image available\*\*

**METHOD FOR SCREENING CRYSTALLIZATION CONDITIONS IN SOLUTION CRYSTAL GROWTH**  
**PROCEDE DE CRIBLAGE DES CONDITIONS DE CRISTALLISATION DANS UNE SOLUTION DE**  
**TIRAGE D'UN CRISTAL**

Patent Applicant/Assignee:

UNIVERSITY OF ALABAMA AT BIRMINGHAM RESEARCH FOUNDATION, 1120  
Administration Building, 701 20th Street South, Birmingham, AL  
35294-0111, US, US (Residence), US (Nationality), (For all designated  
states except: US)

Patent Applicant/Inventor:

DELUCAS Lawrence J, 2739 Altadena Road, Birmingham, AL 35243, US, US  
(Residence), US (Nationality), (Designated only for: US)  
BRAY Terry L, 4016 Bent River Lane, Birmingham, AL 35216, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

GIBBS Barbara S, Harness, Dickey & Pierce, P.L.C., P.O. Box 828,  
Bloomfield Hills, MI 48303, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200060345 A1 20001012 (WO 0060345)

Application: WO 2000US8977 20000405 (PCT/WO US0008977)

Priority Application: US 99128018 19990406

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW  
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG  
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW  
(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-031/00

International Patent Class: G01N-033/00

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims



Fulltext Word Count: 5591

English Abstract

A method of screening protein crystal growth conditions with picogram to microgram amounts of protein in picoliter or nanoliter volumes is provided. A preferred method comprises a **microarray** with a plurality of micro-chambers in the **microarray**. A protein solution is placed into the micro-chambers by an automated dispensing mechanism. The protein crystal growth conditions of each of the micro-chambers is adjusted so that the protein crystal growth conditions in at least two of the micro-chambers differs. Crystallization of the protein solution in the micro-chambers is effected. Protein crystal growth in the micro-chambers is then observed.

French Abstract

La presente invention concerne un procede de criblage des conditions de tirage de cristaux de proteine en des quantites de picogrammes a microgrammes de proteine en des volumes de picolitres ou nanolitres. Dans un mode de realisation prefere, le procede comprend un jeu ordonne de microechantillons avec une pluralite de micro-cavites dans le jeu ordonne de microechantillons. Une solution de proteine est placee dans les micro-cavites au moyen d'un mecanisme de distribution automatise. Les conditions de tirage de cristaux de proteine dans chaque micro-cavite sont reglees de sorte que les conditions de tirage de cristaux de proteine sont differentes dans au moins deux micro-cavites. La cristallisation de la solution de proteine est effectuee dans les micro-cavites. On observe ensuite le tirage de cristaux de proteine.

Legal Status (Type, Date, Text)

Publication 20001012 A1 With international search report.

Examination 20010215 Request for preliminary examination prior to end of 19th month from priority date

16/5/3

DIALOG(R) File 349:PCT Fulltext

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00729283

**A REAGENT SYSTEM AND METHOD FOR INCREASING THE LUMINESCENCE OF LANTHANIDE(III) MACROCYCLIC COMPLEXES**  
**SYSTEME REACTIF ET PROCEDE D'AUGMENTATION DE LA LUMINESCENCE DE COMPLEXES MACROCYCLIQUES LANTHANIDIQUES (III)**

Patent Applicant/Inventor:

LEIF Robert C, 5648 Toyon Road, San Diego, CA 92115-1022, US, US  
(Residence), US (Nationality)

VALLARINO Lidia, 1009 West Avenue, Richmond, VA 23220, US, US (Residence)  
, US (Nationality)

Legal Representative:

SCHWARTZ Robert M, Robert M. Schwartz, P.A., Suite 1125, 169 E. Flagler Street, Miami, FL 33131-1205, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200042048 A1 20000720 (WO 0042048)

Application: WO 2000US1211 20000118 (PCT/WO US0001211)

Priority Application: US 99116316 19990119

Designated States: CA CH DE FI GB JP US

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

Main International Patent Class: C07F-005/00

International Patent Class: C07D-225/00; C07D-255/02

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19305

English Abstract

Disclosed are a spectrofluorimetrically detectable luminescent composition and processes for enhancing the luminescence of one or more lanthanide-containing macrocycles. The luminescent composition comprises

a micelle-producing amount of at least one surfactant, at least one energy transfer acceptor lanthanide element macrocycle compound having an emission spectrum peak in the range from 500 to 950 nanometers, and a luminescence-enhancing amount of at least one energy transfer donor compound of yttrium or a 3-valent lanthanide element having atomic number 59-71, provided that the lanthanide element of said macrocycle compound and the lanthanide element of said energy transfer donor compound are not identical. The addition of gadolinium(III) in the presence of other solutes to both the prototype and the difunctionalized europium, samarium, and terbium macrocyclic complexes, which were taught in our U.S. patents #5,373,093 and #5,696,240, enhances their luminescence. Similar enhancements of luminescence also results for the mono-functionalized europium, samarium, and terbium macrocyclic complexes, which were taught in our U.S. patent #5,696,240. The enhanced luminescence afforded by the composition enables the detection and/or quantitation of many analytes in low concentrations without the use of expensive, complicated time-gated detection systems.

#### French Abstract

La presente invention concerne une composition luminescente detectable par spectrofluorimetrie ainsi que des procedes d'augmentation de la luminescence d'un ou de plusieurs macrocycles a base lanthanidique. Cette composition luminescente comprend une quantite de production micellaire d'au moins un tensioactif, au moins un compose macrocyclique d'element lanthanidique accepteur de transfert energetique presentant un pic de spectre d'emission compris entre 500 et 950 nanometres, et un niveau d'augmentation de luminescence d'au moins un compose de yttrium donneur de transfert energetique ou un element lanthanidique trivalent ayant un nombre atomique compris entre 59 et 71, sous reserve que l'element lanthanidique du compose macrocyclique et l'element lanthanidique du compose donneur de transfert energetique ne soient pas identiques. L'ajout de gadolinium (III) en presence d'autres solutes a la fois dans les complexes macrocycliques prototypes et defonctionnalises comme l'euporium, le samarium, et le terbium, decrits dans les brevets US Ndegrees 5,373,093 et 5,696,240, a pour effet d'augmenter leur luminescence. En outre, on peut obtenir une luminescence similaire avec les complexes macrocycliques mono-fonctionnalises comme l'euporium, le samarium, et le terbium, decrits dans le brevet US. Ndegrees 5,696,240. La luminescence augmentee obtenue grace a cette composition permet de detecter et/ou de quantifier plusieurs analytes dans de faibles concentrations sans avoir a utiliser des systemes de detection a resolution temporelle compliques et chers.

Legal Status (Type, Date, Text)

Publication 20000720 A1 With international search report.

Examination 20001102 Request for preliminary examination prior to end of 19th month from priority date

16/5/4

DIALOG(R) File 349:PCT Fulltext

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00637703

**ANALYTE ASSAY USING PARTICULATE LABELS**

**TITRAGE D'ANALYTES UTILISANT DES MARQUEURS PARTICULAIRES**

Patent Applicant/Assignee:

GENICON SCIENCES CORPORATION, GENICON SCIENCES CORPORATION, 6450-E106 Lusk Boulevard, San Diego, CA 92121, US

Inventor(s):

YGUERABIDE Juan, YGUERABIDE, Juan, 9505 Poole Street, La Jolla, CA 92037, US

YGUERABIDE Evangelina E, YGUERABIDE, Evangelina, E., 9505 Poole Street, La Jolla, CA 92037, US

KOHNE David E, KOHNE, David, E., 364 Nautilus Street, La Jolla, CA 92037, US

JACKSON Jeffrey T, JACKSON, Jeffrey, T., 12738 Casa Avenida, Poway, CA 92064, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9920789 A1 19990429  
Application: WO 98US23160 19981016 (PCT/WO US9823160)  
Priority Application: US 97953713 19971017  
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN  
MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN YU GH  
GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES  
FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN  
TD TG  
Main International Patent Class: C12Q-001/00;  
International Patent Class: C12Q-001/70; G01N-033/53; G01N-033/536;  
G01N-033/532; G01N-031/00; G01N-021/62; C12P-019/34; C07H-019/00;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 78900

#### English Abstract

Method for specific detection of one or more analytes in a sample. The method includes specifically associating any one or more analytes in the sample with a scattered-light detectable particle, illuminating any particle associated with the analytes with light under conditions which produce scattered light from the particle and in which light scattered from one or more particles can be detected by a human eye with less than 500 times magnification and without electronic amplification. The method also includes detecting the light scattered by any such particles under those conditions as a measure of the presence of the analytes.

#### French Abstract

L'invention concerne un procede de detection specifique d'un ou de plusieurs analytes dans un echantillon. Le procede comporte les etapes consistant a associer de maniere specifique un ou plusieurs analytes presents dans l'echantillon a une particule detectable a lumiere diffusee, eclaire une particule associee aux analytes au moyen de lumiere dans des conditions qui permettent la production de lumiere diffusee provenant de la particule ainsi que la detection de lumiere diffusee provenant d'une ou de plusieurs particules, a l'oeil humain avec un grossissement inferieur a x 500 et sans amplification electronique. Le procede comprend egalement la detection de la lumiere diffusee par une de ces particules dans les conditions citees comme mesure de la presence des analytes.

16/5/5

DIALOG(R)File 349:PCT Fulltext  
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00538754

#### ANALYTE ASSAY USING PARTICULATE LABELS

#### DETECTION D'ANALYTES A L'AIDE DE MARQUEURS PARTICULAIRES

Patent Applicant/Assignee:

SPECTRAMETRIX INC, SPECTRAMETRIX INC. , 6450 &shy; E106 Lusk Boulevard,  
San Diego, CA 92121 , US

Inventor(s):

Yguerabide Evangelina E, Yguerabide, Evangelina, E. , 9505 Poole Street,  
La Jolla, CA 92037 , US

Kohne David E, Kohne, David, E. , 364 Nautilus Street, La Jolla, CA 92037  
, US

Jackson Jeffrey T, Jackson, Jeffrey, T. , 12738 Casa Avenida, Poway, CA  
92064 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9740181 A1 19971030

Application: WO 97US6584 19970417 (PCT/WO US9706584)

Priority Application: US 9616383 19960425

Designated States: AT AU BR CA CH CN CZ DE DK ES FI GB HU IL IS JP KP KR LU  
MX NO NZ PL PT RO RU SE SG US AT BE CH DE DK ES FI FR GB GR IE IT LU MC  
NL PT SE

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International Patent Class: G03G-005/00;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
    Detailed Description  
    Claims  
Fulltext Word Count: 61788

English Abstract

Method for specific detection of one or more analytes in a sample. The method includes specifically associating any one or more analytes in the sample with a scattered&shy;light detectable particle, illuminating any particle associated with the analytes with light under conditions which produce scattered light from the particle and in which light scattered from one or more particles can be detected by a human eye with less than 500 times magnification and without electronic amplification. The method also includes detecting the light scattered by any such particles under those conditions as a measure of the presence of the analytes.

French Abstract

Procede permettant la detection specifique d'un ou plusieurs analytes dans un echantillon, qui consiste a associer specifiquement un ou plusieurs analytes presents dans l'echantillon a une particule detectable par la dispersion de lumiere, et a eclairer toute particule associee aux analytes avec de la lumiere dans des conditions permettant aux particules de produire une lumiere dispersee et dans lesquelles la lumiere dispersee par une ou plusieurs particules peut etre detectee par l'oeil humain a une amplification inferieure a 500 fois et sans amplification electronique. Ledit procede consiste egalement a detecter la lumiere dispersee par l'une quelconque de ces particules dans lesdites conditions pour permettre d'identifier la presence des analytes.

?s enzyme(n20)assay(n20)microarray  
    50388 ENZYME  
    48707 ASSAY  
    785 MICROARRAY  
S17 5 ENZYME(N20)ASSAY(N20)MICROARRAY  
?t 17/5/1-5

Estimated cost of output requested is: \$25.50  
Are you ready to receive all output? (Yes/No/Help)  
?y

17/5/1  
DIALOG(R)File 349:PCT Fulltext  
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00748426   \*\*Image available\*\*

REAGENTS AND METHODS FOR IDENTIFYING AND MODULATING EXPRESSION OF GENES  
REGULATED BY p21

REACTIFS ET PROCEDES D'IDENTIFICATION, ET DE MODULATION D'EXPRESSION, DU  
GENE REGULE PAR p21

Patent Applicant/Assignee:

BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS, 352 Henry Administration  
Building, 506 South Wright Street, Urbana, IL 61801, US, US (Residence)  
, US (Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

CHANG Bey-Dih, 1116 Cambria Lane, Lombard, IL 60148, US, US (Residence),  
-- (Nationality), (Designated only for: US)

RONINSON Igor B, 2731 Lincoln Lane, Wilmette, IL 60091, US, US  
(Residence), US (Nationality), (Designated only for: US)

Legal Representative:

NOONAN Kevin E, McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker  
Drive, Chicago, IL 60606, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200061751 A1 20001019 (WO 0061751)

Application: WO 2000US9286 20000407 (PCT/WO US0009286)

Priority Application: US 99128676 19990409; US 99449589 19991129

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE

ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT  
UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

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International Patent Class: C07K-014/47; G01N-033/68

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 26048

#### English Abstract

This invention provides methods and reagents for identifying genes involved in cell cycle progression, growth promotion, modulation of apoptosis, cellular senescence and aging, and methods for identifying compounds that inhibit or potentiate cellular senescence, regulated by p21.

#### French Abstract

L'invention concerne des procedes et reactifs d'identification de genes impliquees dans la progression du cycle cellulaire, le developpement de la croissance, la modulation de l'apoptose, la senescence et le vieillissement des cellules; elle concerne egalement des procedes d'identification de composees inhibant ou potentialisant la senescence des cellules regulee par p21.

Legal Status (Type, Date, Text)

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Examination 20001116 Request for preliminary examination prior to end of 19th month from priority date

17/5/2

DIALOG(R)File 349:PCT Fulltext

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00747851

**QUANTITATIVE ASSAY FOR EXPRESSION OF GENES IN MICROARRAY**

**BIOANALYSE QUANTITATIVE D'EXPRESSION DE GENES DANS DES MICROECHANTILLONS**

Patent Applicant/Assignee:

SIR MORTIMER B DAVIS JEWISH GENERAL HOSPITAL, 3755 Chemin de la Cote Ste-Catherine Road, Montreal, Quebec H3T 1E2, CA, CA (Residence), CA (Nationality)

Patent Applicant/Inventor:

WANG Eugenia, 3755 Chemin de la Cote Ste-Catherine Road, Montreal, Quebec H3T 1E2, CA, CA (Residence), CA (Nationality)

Legal Representative:

PABST Patrea L, Arnall Golden & Gregory, LLP, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA 30309-3450, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200060126 A2 20001012 (WO 0060126)

Application: WO 2000US9526 20000410 (PCT/WO US0009526)

Priority Application: CA 2268695 19990408; US 99129233 19990414; US 99299193 19990423

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK

DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ

TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12Q-001/68

Publication Language: English

Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 14178

English Abstract

A method has been developed for detection of gene expression or hybridization in microarrays, for example, in combinatorial libraries where quantities are very small and spots located very closely, resulting in uncomfortable situations where intense reaction can spill over into the adjacent spots, and therefore obscure the accuracy of the reaction of the neighboring sites. The **assay** uses a digoxigenin **enzyme assay** for detection. A method for enhancing the reliability of analysis of expression of DNA in **microarray** formats has also been developed, using software analysis that normalizes the spots. This process uses deformable template techniques to quantify large-scale array data automatically, despite possible spatial distortion of the arrays. Each node in the deformable template represents a gene spot, and iterates according to the gradient descent rule, which minimizes an energy function combining data mismatch energy and template deformation energy.

French Abstract

L'invention concerne une methode mise au point pour la detection d'expression ou d'hybridation de genes dans des microechantillons, par exemple dans des bibliotheques combinatoires dans lesquelles les quantites sont minimales et les emplacements tres rapproches, pouvant provoquer des situations indesirables dans lesquelles une reaction intense peut deborder sur les emplacements adjacents et, par consequent, fausser l'exactitude de la reaction des sites voisins. On utilise dans cette bioanalyse un test d'activite enzymatique de digoxigenine pour la detection. L'invention concerne egalement une methode mise au point dans le but d'ameliorer la fiabilite de l'analyse de l'expression d'ADN dans des microechantillons, par analyse informatique destinee a normaliser les emplacements. Ce procede fait appel a des techniques basees sur des modeles adaptables permettant de quantifier automatiquement des donnees d'echantillons a grande echelle, en depit d'une possible distorsion spatiale des echantillons. Chaque noeud du modele adaptable represente l'emplacement d'un gene, et se repete selon la regle de descente de gradient, minimisant une fonction energie combinant l'energie de desadaptation des donnees et l'energie d'adaptation du modele.

Legal Status (Type, Date, Text)

Publication 20001012 A2 Without international search report and to be republished upon receipt of that report.  
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17/5/3

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00731651 \*\*Image available\*\*

COMPOSITION AND METHOD FOR IN VIVO AND IN VITRO ATTENUATION OF GENE  
EXPRESSION USING DOUBLE STRANDED RNA  
COMPOSITION ET METHODE DESTINEES A L'ATTENUATION IN VIVO ET IN VITRO DE  
L'EXPRESSION GENIQUE UTILISANT DE L'ARN DOUBLE BRIN

Patent Applicant/Assignee:

MEDICAL COLLEGE OF GEORGIA RESEARCH INSTITUTE INC, 1120 15th Street, Room  
CB 1810, Augusta, GA 30912-4810, US, US (Residence), US (Nationality)

Patent Applicant/Inventor:

LI Yin-Xiong, 1120 15th Street, Room CB 1810, Augusta, GA 30912-4810, US,  
US (Residence), US (Nationality)  
FARRELL Michael J, 1011 Hickman Road, Augusta, GA 30904, US, US  
(Residence), US (Nationality)  
KIRBY Margaret L, 7043 Laramie Avenue, Canoga Park, CA 91306, US, US  
(Residence), US (Nationality)

Legal Representative:

SANDBERG Victoria A, Muetting, Raasch & Gebhardt, P.A., P.O. Box 581415,  
Minneapolis, MN 55458-1415, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200044914 A1 20000803 (WO 0044914)

Application: WO 2000US2227 20000128 (PCT/WO US0002227)

Priority Application: US 99117635 19990128; US 2000175440 20000111

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RQ RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12N-015/63

International Patent Class: A01K-067/027; A61K-031/713

Publication Language: English

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Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 13975

#### English Abstract

Introduction of double stranded RNA into cells, cell culture, organs and tissues, and whole organisms, particularly vertebrates, specifically attenuates gene expression.

#### French Abstract

La presente invention concerne l'introduction d'ARN double brin dans des cellules, des cultures de cellules, des organes et des tissus, et dans des organismes entiers, en particulier de vertebres, qui permet d'attenuer specifiquement l'expression genique.

Legal Status (Type, Date, Text)

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Claim Mod 20000914 Later publication of amended claims under Article 19 received: 20000717

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00715211 \*\*Image available\*\*

#### EMBRYONIC STEM CELLS

#### CELLULES SOUCHES EMBRYONNAIRES

Patent Applicant/Assignee:

MONASH UNIVERSITY, Clayton Road, Clayton, VIC 3168, AU, AU (Residence),  
AU (Nationality), (For all designated states except: US)

NATIONAL UNIVERSITY OF SINGAPORE, National University Hospital, Lower  
Kent Ridge Road, Singapore, SG, SG (Residence), SG (Nationality), (For  
all designated states except: US)

HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT CO LTD, The Volcani  
Center, 50250 Bet Dagan, IL, IL (Residence), IL (Nationality), (For all  
designated states except: US)

Patent Applicant/Inventor:

REUBINOFF Benjamin Eithan, 26 Oswald Street, Elsternwick, VIC 3185, AU,  
AU (Residence), IL (Nationality), (Designated only for: US)

PERA Martin Frederick, 53 Highbury Grove, Prahran, VIC 3181, AU, AU  
(Residence), US (Nationality), (Designated only for: US)

YEE Fong Chui, 16 West Coast Crescent #01-10, Singapore 128044, SG, SG  
(Residence), SG (Nationality), (Designated only for: US)

TROUNSON Alan Osborne, 25 Beatty Crescent, Ashburton, VIC 3147, AU, AU

(Residence), AU (Nationality), (Designated only for: US)  
BONGSO Arifteen, Tower C, 32 Dover Rise, #02-03 DoverPark View, Singapore  
1368686, SG, SG (Residence), SG (Nationality), (Designated only for:  
US)

Legal Representative:

PHILLIPS ORMONDE & FITZPATRICK, 367 Collins Street, Melbourne, VIC 3000,  
AU

Patent and Priority Information (Country, Number, Date):

Patent: WO 200027995 A1 20000518 (WO 0027995)

Application: WO 99AU990 19991109 (PCT/WO AU9900990)

Priority Application: AU 987009 19981109; AU 992852 19990915

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR

LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ  
TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: C12N-005/00

International Patent Class: C12N-005/08

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 12546

English Abstract

The present invention relates to undifferentiated human embryonic stem cells, methods of cultivation and propagation, production of differentiated cells and in particular the production of human ES capable of yielding somatic differentiated cells in vitro, as well as committed progenitor cells capable of giving rise to mature somatic cells and uses thereof. In one aspect of the present invention, there is provided a purified preparation of undifferentiated human embryonic stem cells capable of proliferation in vitro. In another aspect, there is provided a somatic cell differentiated in vitro from an undifferentiated embryonic stem cell. There is also provided a committed progenitor cell capable of giving rise to mature somatic cells.

French Abstract

Cette invention se rapporte a des cellules souches embryonnaires humaines non différenciées, a des procédés de culture et de propagation, a la production de cellules différenciées et en particulier a la production d'une souche embryonnaire (ES) humaine capable de fournir des cellules différenciées somatiques in vitro, ainsi que des cellules progéniteurs engagés capables de faire naître des cellules somatiques matures, ainsi qu'aux utilisations de celles-ci. Dans l'un de ses aspects, cette invention propose une préparation purifiée de cellules souches embryonnaires humaines non différenciées capables de prolifération in vitro. Dans un autre de ses aspects, cette invention propose une cellule somatique différenciée in vitro a partir d'une cellule souche embryonnaire non différenciée. Cette invention concerne également une cellule progéniteur engagée capable de faire naître des cellules somatiques matures.

Legal Status (Type, Date, Text)

Publication 20000518 A1 With international search report.

Examination 20000629 Request for preliminary examination prior to end of  
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17/5/5

DIALOG(R) File 349:PCT.Fulltext.

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00649489

GENETIC INHIBITION BY DOUBLE-STRANDED RNA



**INHIBITION GENETIQUE PAR DE L'ARN DOUBLE BRIN**

Patent Applicant/Assignee:

THE CARNEGIE INSTITUTE OF WASHINGTON, THE CARNEGIE INSTITUTE OF  
WASHINGTON, 1530 P Street, N.W., Washington, DC 20005, US  
THE UNIVERSITY OF MASSACHUSETTS, THE UNIVERSITY OF MASSACHUSETTS, One  
Beacon Street, Boston, MA 02108, US

Inventor(s):

FIRE Andrew, FIRE, Andrew, 2320 Bright Leaf Way, Baltimore, MD 21210, US  
XU Sigun, XU, Sigun, 1755 Warminton Court, Ballwin, MO 63021, US  
MONTGOMERY Mary K, MONTGOMERY, Mary, K., 233 Macalester Street, St. Paul,  
MN 55105, US  
KOSTAS Stephen A, KOSTAS, Stephen, A., 126 East Melrose Avenue,  
Baltimore, MD 21212, US  
TIMMONS Lisa, TIMMONS, Lisa, 2408 Brambleton Road, Baltimore, MD 21209,  
US  
TABARA Hiroaki, TABARA, Hiroaki, Apartment &1, 145 Orient Street,  
Worcester, MA 01604, US  
DRIVER Samuel E, DRIVER, Samuel, E., Apartment &4, 1714 Commonwealth  
Avenue, Brighton, MA 02135, US  
MELLO Craig C, MELLO, Craig, C., 19 Ryan Road, Shrewsbury, MA 01545, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9932619 A1 19990701

Application: WO 98US27233 19981221 (PCT/WO US9827233)

Priority Application: US 9768562 19971223

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU  
LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA  
UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM  
AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM  
GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/11;

International Patent Class: C12N-015/63; C12N-015/82;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 14720

**English Abstract**

A process is provided of introducing an RNA into a living cell to inhibit gene expression of a target gene in that cell. The process may be practiced < i> ex vivo< /i> or < i> in vivo< /i> . The RNA has a region with double-stranded structure. Inhibition is sequence-specific in that the nucleotide sequences of the duplex region of the RNA and of a portion of the target gene are identical. The present invention is distinguished from prior art interference in gene expression by antisense or triple-strand methods.

**French Abstract**

L'invention porte sur un procede pouvant s'operer < i> ex vivo< /i> ou < i> in vivo< /i> consistant a introduire de l'ARN dans une cellule vivante pour inhiber l'expression genique d'un gene cible de cette cellule. L'ARN possede une region a structure double brin. L'inhibition est specifique d'une sequence en ce sens que les sequences nucleotidiques de la region double de l'ARN et d'une partie du gene cible sont identiques. Ladite invention differe des interferences avec l'expression genique des techniques anterieures par l'utilisation de procedes antisens ou a triple brin.

?s enzyme? ?/cm and assay? ?/cm and microarray? ?/cm

15325 ENZYME? ?/CM

9249 ASSAY? ?/CM

167 MICROARRAY? ?/CM

S18

76 ENZYME? ?/CM AND ASSAY? ?/CM AND MICROARRAY? ?/CM

?s enzyme? ?cm (n20) assay? ?/cm (n20) microarray? ?/cm

0 ENZYME? ?CM/CM

9249 ASSAY? ?/CM

167 MICROARRAY? ?/CM

S19

0 ENZYME? ?CM (N20) ASSAY? ?/CM (N20) MICROARRAY? ?/CM

?s enzyme? ?/cm (n20) assay? ?/cm (n20) microarray? ?/cm  
15325 ENZYME? ?/CM  
9249 ASSAY? ?/CM  
167 MICROARRAY? ?/CM  
S20 0 ENZYME? ?/CM (N20) ASSAY? ?/CM (N20) MICROARRAY? ?/CM  
?s (peptide? ?/cm or protein? ?/cm) (n20) assay? ?/cm (n20) MICROARRAY? ?/CM  
21855 PEPTIDE? ?/CM  
29305 PROTEIN? ?/CM  
9249 ASSAY? ?/CM  
167 MICROARRAY? ?/CM  
S21 43 (PEPTIDE? ?/CM OR PROTEIN? ?/CM) (N20) ASSAY? ?/CM (N20)  
MICROARRAY? ?/CM  
?T 21/5/1

21/5/1  
DIALOG(R) File 349:PCT Fulltext  
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00741537

**UPA, A UNIVERSAL PROTEIN ARRAY SYSTEM**  
**SYSTEME DE GROUPEMENT UNIVERSEL DE PROTEINES (UPA)**  
Patent Applicant/Assignee:

THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE  
SECRETARY DEPARTMENT OF HEALTH AND HUMAN SERVICES THE NATIONAL  
INSTITUTES OF HEALTH, Office of Technology Transfer, Suite #325, 6011  
Executive Boulevard, Rockville, MD 20852, US, US (Residence), US  
(Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

GE Hui, 437 Upshire Circle, Gaithersburg, MD 20878, US, US (Residence),  
CN (Nationality), (Designated only for: US)

Legal Representative:

NOONAN William D, Klarquist, Sparkman, Campbell, Leigh & Whinston, LLP,  
Suite 1600, One World Trade Center, 121 SW Salmon Street, Portland, OR  
97204, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200054046 A2 20000914 (WO 0054046)

Application: WO 2000US6244 20000310 (PCT/WO US0006244)

Priority Application: US 99123586 19990310

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK  
DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK

LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL  
TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-033/48

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 16583

**English Abstract**

This invention relates to ordered arrays of molecules, for instance polypeptides or proteins. Such arrays, referred to as universal protein arrays, are described in both macro- and microarray formats. Methods of production and use of such arrays are also described. Also disclosed are kits for the use of such arrays and the preparation of probes for use with them.

**French Abstract**

L'invention a trait a des groupements ordonnes de molecules, par exemple de polypeptides ou de proteines. Ces groupements, appeles groupements universels de proteines, sont decrits dans des formats de macrogroupements et de microgroupements. Des procedes de production et d'utilisation de ces groupements sont egalement decrits. L'invention concerne egalement des troussees utiles pour ces groupements et pour la

preparation de sondes s'utilisant avec ceux-ci.

Legal Status (Type, Date, Text)

Publication 20000914 A2 Without international search report and to be republished upon receipt of that report.

Examination 20001130 Request for preliminary examination prior to end of 19th month from priority date

Search Rpt 20001221 Late publication of international search report

?S S21 and (glycerol? or polyethylene()glycol?)

43 S21

34003 GLYCEROL?

65081 POLYETHYLENE

67208 GLYCOL?

30946 POLYETHYLENE(W)GLYCOL?

S22 42 S21 AND (GLYCEROL? OR POLYETHYLENE()GLYCOL?)

?s s22 and (dot? or microdot? or spot?)

42 S22

62567 DOT?

59 MICRODOT?

30954 SPOT?

S23 42 S22 AND (DOT? OR MICRODOT? OR SPOT?)

?s s23 and (three()dimension? or 3()dimension?)

42 S23

220621 THREE

143179 DIMENSION?

25305 THREE(W)DIMENSION?

492705 3

143179 DIMENSION?

3359 3(W)DIMENSION?

S24 39 S23 AND (THREE()DIMENSION? OR 3()DIMENSION?)

?t 24/5/1

24/5/1

DIALOG(R)File 349:PCT Fulltext

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00648172 \*\*Image available\*\*

**UBIQUITIN-LIKE CONJUGATING PROTEIN**

**PROTEINE DE CONJUGAISON DU TYPE UBIQUITINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12, Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9931252 A1 19990624

Application: WO 98US25564 19981202 (PCT/WO US9825564)

Priority Application: US 97989289 19971212

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/52;

International Patent Class: C12N-005/10; C12N-001/21; C12N-009/00;

C12Q-001/68; C07K-016/40; A61K-038/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19213

# English Abstract

The invention provides a human ubiquitin-like conjugating protein (UBCLE) and polynucleotides which identify and encode UBCLE. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of UBCLE.

# French Abstract

Cette invention se rapporte a une proteine de conjugaison du type ubiquitine humaine (UBCLE) et a des polynucleotides qui identifient et codent cette proteine (UBCLE). Cette invention presente egalement des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes, ainsi que des procedes pour traiter ou prevenir des troubles associes a l'expression de cette proteine (UBCLE).

?ds

Set	Items	Description
S1	1134	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) AND (ARRAY? ?/CM OR MICRO-ARRAY? ?/CM)
S2	401	S1 AND POLYETHYLENE()GLYCOL
S3	0	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N10) MICROARRAY? ?/CM (N-10) POLYETHYLENE()GLYCOL
S4	19	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) MICROARRAY? ?/CM
S5	73	S2 AND (AEROSOL? OR MIST? OR NEBULI?)
S6	67	S5 AND BIOLOG?
S7	229	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) (DOT? ?/CM OR SPOT? ?/CM OR MICRODOT? ?/CM)
S8	133	S7 AND (GLYCEROL OR POLYETHYLENE()GLYCOL)
S9	46	S8 AND (ARRAY OR MICROARRAY)
S10	21	S9 AND CHIP
S11	20	S10 AND SCAN?
S12	20	S11 AND (FLUOR? OR CHROM?)
S13	2	S12 AND (MIST? OR AEROSOL? OR NEBULI?)
S14	120	(PROTEIN? ?/CM OR PEPTID? ?/CM) AND (MICROARRAY? ?/AB OR M-ICROARRAY? ?/CM)
S15	190	S14 AND (5()MICRON?) OR (FIVE()MICRON)
S16	5	S14 AND (5()MICRON? OR FIVE()MICRON?)
S17	5	ENZYME(N20)ASSAY(N20)MICROARRAY
S18	76	ENZYME? ?/CM AND ASSAY? ?/CM AND MICROARRAY? ?/CM
S19	0	ENZYME? ?CM (N20) ASSAY? ?/CM (N20) MICROARRAY? ?/CM
S20	0	ENZYME? ?/CM (N20) ASSAY? ?/CM (N20) MICROARRAY? ?/CM
S21	43	(PEPTIDE? ?/CM OR PROTEIN? ?/CM) (N20) ASSAY? ?/CM (N20) M-ICROARRAY? ?/CM
S22	42	S21 AND (GLYCEROL? OR POLYETHYLENE()GLYCOL?)
S23	42	S22 AND (DOT? OR MICRODOT? OR SPOT?)
S24	39	S23 AND (THREE()DIMENSION? OR 3()DIMENSION?)
?s s24 and print?		
	39	S24
	52496	PRINT?
S25	39	S24 AND PRINT?
?s s24 and hemispher?		
	39	S24
	6020	HEMISPHER?
S26	0	S24 AND HEMISPHER?
?s s24 and droplet?		
	39	S24
	13103	DROPLET?
S27	0	S24 AND DROPLET?
?s s24 and discrete		
	39	S24
	41265	DISCRETE
S28	0	S24 AND DISCRETE
?s s24 and cross()contam?		
	39	S24
	180862	CROSS
	55648	CONTAM?
	1761	CROSS(W)CONTAM?
S29	0	S24 AND CROSS()CONTAM?
?s s24 and microarray? ?/ab		

39 S24  
48 MICROARRAY? ?/AB  
S30 0 S24 AND MICROARRAY? ?/AB  
?s (peptide? ?/cm or protein? ?/cm or enzyme? ?/cm) (n20) assay? ?/cm (n20) (microarray  
? ?/cm or microarray? ?/ab)  
21855 PEPTIDE? ?/CM  
29305 PROTEIN? ?/CM  
15325 ENZYME? ?/CM  
9249 ASSAY? ?/CM  
167 MICROARRAY? ?/CM  
48 MICROARRAY? ?/AB  
S31 43 (PEPTIDE? ?/CM OR PROTEIN? ?/CM OR ENZYME? ?/CM) (N20)  
ASSAY? ?/CM (N20) (MICROARRAY? ?/CM OR MICROARRAY? ?/AB)  
?t 31/5/1

31/5/1  
DIALOG(R)File 349:PCT Fulltext  
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00741537

**UPA, A UNIVERSAL PROTEIN ARRAY SYSTEM**  
**SYSTEME DE GROUPEMENT UNIVERSEL DE PROTEINES (UPA)**

Patent Applicant/Assignee:

THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE  
SECRETARY DEPARTMENT OF HEALTH AND HUMAN SERVICES THE NATIONAL  
INSTITUTES OF HEALTH, Office of Technology Transfer, Suite #325, 6011  
Executive Boulevard, Rockville, MD 20852, US, US (Residence), US  
(Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

GE Hui, 437 Upshire Circle, Gaithersburg, MD 20878, US, US (Residence),  
CN (Nationality), (Designated only for: US)

Legal Representative:

NOONAN William D, Klarquist, Sparkman, Campbell, Leigh & Whinston, LLP,  
Suite 1600, One World Trade Center, 121 SW Salmon Street, Portland, OR  
97204, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 200054046 A2 20000914 (WO 0054046)

Application: WO 2000US6244 20000310 (PCT/WO US0006244)

Priority Application: US 99123586 19990310

Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK

DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK

LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL

TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

(AP) GH GM KE LS MW SD SL SZ TZ UG ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Main International Patent Class: G01N-033/48

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 16583

**English Abstract**

This invention relates to ordered arrays of molecules, for instance polypeptides or proteins. Such arrays, referred to as universal protein arrays, are described in both macro- and microarray formats. Methods of production and use of such arrays are also described. Also disclosed are kits for the use of such arrays and the preparation of probes for use with them.

**French Abstract**

L'invention a trait a des groupements ordonnes de molecules, par exemple de polypeptides ou de proteines. Ces groupements, appeles groupements universels de proteines, sont decrits dans des formats de macrogroupements et de microgroupements. Des procedes de production et d'utilisation de ces groupements sont egalement decrits. L'invention

concerne également des troussees utiles pour ces groupements et pour la preparation de sondes s'utilisant avec ceux-ci.

Legal Status (Type, Date, Text)

Publication 20000914 A2 Without international search report and to be republished upon receipt of that report.  
Examination 20001130 Request for preliminary examination prior to end of 19th month from priority date  
Search Rpt 20001221 Late publication of international search report  
?s s31 and (glycerol or polyethylene()glycol)  
43 S31  
33632 GLYCEROL  
65081 POLYETHYLENE  
56798 GLYCOL  
27239 POLYETHYLENE(W)GLYCOL  
S32 42 S31 AND (GLYCEROL OR POLYETHYLENE()GLYCOL)  
?t 31/5/2

31/5/2

DIALOG(R)File 349:PCT Fulltext

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00648172 \*\*Image available\*\*

**UBIQUITIN-LIKE CONJUGATING PROTEIN**

**PROTEINE DE CONJUGAISON DU TYPE UBIQUITINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12, Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9931252 A1 19990624

Application: WO 98US25564 19981202 (PCT/WO US9825564)

Priority Application: US 97989289 19971212

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/52;

International Patent Class: C12N-005/10; C12N-001/21; C12N-009/00; C12Q-001/68; C07K-016/40; A61K-038/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19213

English Abstract

The invention provides a human ubiquitin-like conjugating protein (UBCLE) and polynucleotides which identify and encode UBCL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of UBCL.

French Abstract

Cette invention se rapporte a une proteine de conjugaison du type ubiquitine humaine (UBCL) et a des polynucleotides qui identifient et codent cette proteine (UBCL). Cette invention presente egalement des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes, ainsi que des procedes pour traiter ou prevenir des troubles associes a l'expression de cette proteine (UBCL).

?t 31/5/3

31/5/3

DIALOG(R)File 349:PCT Fulltext  
(c) 2001 WIPO/MicroPat. All rts. reserv.

00648151 \*\*Image available\*\*

# HUMAN PHOSPHATASES

## PHOSPHATASES HUMAINES

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter  
Drive, Palo Alto, CA 94304, US

Inventor(s):

SHAH Purvi, SHAH, Purvi, 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087, US

HILLMAN Jennifer L, HILLMAN, Jennifer, L., 230 Monroe Drive &12, Mountain  
View, CA 94040, US

CORLEY Neil C, CORLEY, Neil, C., 1240 Dale Avenue &30, Mountain View, CA  
94040, US

LAL Preeti, LAL, Preeti, 2382 Lass Drive, Santa Clara, CA 95054, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9931225 A2 19990624

Application: WO 98US25559 19981202 (PCT/WO US9825559)

Priority Application: US 97992035 19971217

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-009/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19601

### English Abstract

The invention provides human phosphatases (HPA) and polynucleotides which identify and encode HPA. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating disorders associated with expression of HPA.

### French Abstract

Cette invention se rapporte a des phosphatases humaines (HPA) et a des polynucleotides qui identifient et codent ces phosphatases HPA. Cette invention presente egalement des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes, ainsi que des procedes pour traiter les troubles associes a l'expression de phosphatases HPA.

?ds

Set	Items	Description
S1	1134	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) AND (ARRAY? ?/CM OR MICRO- ARRAY? ?/CM)
S2	401	S1 AND POLYETHYLENE() GLYCOL
S3	0	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N10) MICROARRAY? ?/CM (N- 10) POLYETHYLENE() GLYCOL
S4	19	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) MICROARRAY? ?/CM
S5	73	S2 AND (AEROSOL? OR MIST? OR NEBULI?)
S6	67	S5 AND BIOLOG?
S7	229	(PROTEIN? ?/CM OR PEPTIDE? ?/CM) (N20) (DOT? ?/CM OR SPOT? ?/CM OR MICRODOT? ?/CM)
S8	133	S7 AND (GLYCEROL OR POLYETHYLENE() GLYCOL)
S9	46	S8 AND (ARRAY OR MICROARRAY)
S10	21	S9 AND CHIP
S11	20	S10 AND SCAN?

S12 20 S11 AND (FLUOR? OR CHROM?)  
 S13 2 S12 AND (MIST? OR AEROSOL? OR NEBULI?)  
 S14 120 (PROTEIN? ?/CM OR PEPTID? ?/CM) AND (MICROARRAY? ?/AB OR M-  
 ICROARRAY? ?/CM)  
 S15 190 S14 AND (5()MICRON?) OR (FIVE()MICRON)  
 S16 5 S14 AND (5()MICRON? OR FIVE()MICRON?)  
 S17 5 ENZYME(N20)ASSAY(N20)MICROARRAY  
 S18 76 ENZYME? ?/CM AND ASSAY? ?/CM AND MICROARRAY? ?/CM  
 S19 0 ENZYME? ?CM (N20) ASSAY? ?/CM (N20) MICROARRAY? ?/CM  
 S20 0 ENZYME? ?/CM (N20) ASSAY? ?/CM (N20) MICROARRAY? ?/CM  
 S21 43 (PEPTIDE? ?/CM OR PROTEIN? ?/CM) (N20) ASSAY? ?/CM (N20) M-  
 ICROARRAY? ?/CM  
 S22 42 S21 AND (GLYCEROL? OR POLYETHYLENE()GLYCOL?)  
 S23 42 S22 AND (DOT? OR MICRODOT? OR SPOT?)  
 S24 39 S23 AND (THREE()DIMENSION? OR 3()DIMENSION?)  
 S25 39 S24 AND PRINT?  
 S26 0 S24 AND HEMISPHER?  
 S27 0 S24 AND DROPLET?  
 S28 0 S24 AND DISCRETE  
 S29 0 S24 AND CROSS()CONTAM?  
 S30 0 S24 AND MICROARRAY? ?/AB  
 S31 43 (PEPTIDE? ?/CM OR PROTEIN? ?/CM OR ENZYME? ?/CM) (N20) ASS-  
 AY? ?/CM (N20) (MICROARRAY? ?/CM OR MICROARRAY? ?/AB)  
 S32 42 S31 AND (GLYCEROL OR POLYETHYLENE()GLYCOL)  
 ?t 31/5/3-43

Estimated cost of output requested is: \$209.10  
 Are you ready to receive all output? (Yes/No/Help)  
 ?y

31/5/3

DIALOG(R)File 349:PCT Fulltext  
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00648151 \*\*Image available\*\*

# **HUMAN PHOSPHATASES**

## **PHOSPHATASES HUMAINES**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter  
 Drive, Palo Alto, CA 94304, US

Inventor(s):

SHAH Purvi, SHAH, Purvi, 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
 94087, US

HILLMAN Jennifer L, HILLMAN, Jennifer, L., 230 Monroe Drive &12, Mountain  
 View, CA 94040, US

CORLEY Neil C, CORLEY, Neil, C., 1240 Dale Avenue &30, Mountain View, CA  
 94040, US

LAL Preeti, LAL, Preeti, 2382 Lass Drive, Santa Clara, CA 95054, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9931225 A2 19990624

Application: WO 98US25559 19981202 (PCT/WO US9825559)

Priority Application: US 97992035 19971217

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C12N-009/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19601

English Abstract

The invention provides human phosphatases (HPA) and polynucleotides which  
 identify and encode HPA. The invention also provides expression vectors,



host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating disorders associated with expression of HPA.

#### French Abstract

Cette invention se rapporte a des phosphatases humaines (HPA) et a des polynucleotides qui identifient et codent ces phosphatases HPA. Cette invention presente egalement des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes, ainsi que des procedes pour traiter les troubles associes a l'expression de phosphatases HPA.

31/5/4

DIALOG(R)File 349:PCT Fulltext

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00648084 \*\*Image available\*\*

#### HUMAN TUMOR NECROSIS FACTOR-R2-LIKE PROTEINS

#### PROTEINES DU TYPE FACTEUR HUMAIN DE NECROSE TUMORALE R2

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter Drive, Palo Alto, CA 94304, US

Inventor(s):

BANDMAN Olga, BANDMAN, Olga, 366 Anna Avenue, Mountain View, CA 94043, US  
HILLMAN Jennifer L, HILLMAN, Jennifer, L., 230 Monroe Drive #12, Mountain View, CA 94040, US

AU-YOUNG Janice, AU-YOUNG, Janice, 1419 Kains Avenue, Berkeley, CA 94702, US

TANG Y Tom, TANG, Y., Tom, 4230 Ranwick Court, San Jose, CA 95118, US

KASER Matthew R, KASER, Matthew, R., 4793 Ewing Road, Castro Valley, CA 94546- 1017, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9931128 A2 19990624

Application: WO 98US25649 19981202 (PCT/WO US9825649)

Priority Application: US 97991945 19971216

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C07K-014/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 21630

#### English Abstract

The invention provides human tumor necrosis factor-R2-like proteins (TR2P) and polynucleotides which identify and encode TR2P. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of TR2P.

#### French Abstract

L'invention porte sur des proteines du type facteur humain de necrose tumorale R2 (TR2P) et sur des polynucleotides codant pour les TR2P, sur des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes, et sur des procedes de traitement ou prevention de troubles associes a l'expression des TR2P.

31/5/5

DIALOG(R)File 349:PCT Fulltext

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00646742    \*\*Image available\*\*

**CYCLIC GMP PHOSPHODIESTERASE  
PHOTODIESTERASE DE GMP CYCLIQUE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

FISHER Douglas A, FISHER, Douglas, A. , 600 Meridian Street &824, Groton,  
CT 06340 , US

GOODING Douglas H, GOODING, Douglas, H. , 937 Junipero Avenue, Redwood  
City, CA 94061 , US

STREETER David Gray, STREETER, David, Gray , 1211 Pinecrest Drive,  
Boulder Creek, CA 95006 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9929873 A1 19990617

Application: WO 98US25756 19981202 (PCT/WO US9825756)

Priority Application: US 97987466 19971209

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/52;

International Patent Class: C12N-009/16; A61K-038/46; C07K-016/40;  
C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19827

**English Abstract**

The invention provides a human cyclic GMP phosphodiesterase (PDE9A) and polynucleotides which identify and encode PDE9A. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of PDE9A.

**French Abstract**

L'invention concerne une photodiesterase de GMP cyclique (PDE9A) et des polynucleotides qui identifient et codent PDE9A. Elle porte aussi sur des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes, ainsi que sur des methodes de traitement ou de prevention de troubles associes a l'expression de PDE9A.

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DIALOG(R) File 349:PCT Fulltext

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00646719

**G-PROTEIN COUPLED RECEPTORS ASSOCIATED WITH IMMUNE RESPONSE  
RECEPTEURS COUPLES A LA PROTEINE G ASSOCIES A UNE REACTION IMMUNITAIRE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

BANDMAN Olga, BANDMAN, Olga , 366 Anna Avenue, Mountain View, CA 94043 ,  
US

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US

YUE Henry, YUE, Henry , 826 Lois Avenue, Sunnyvale, CA 94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9929849 A1 19990617

Application: WO 98US25565 19981202 (PCT/WO US9825565)

Priority Application: US 97988876 19971211

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/705; A61K-038/16; G01N-033/68;  
C12Q-001/68; C07K-016/28;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20938

#### English Abstract

The invention provides two human G-protein coupled receptors associated with immune response (GRIR) and polynucleotides which identify and encode GRIR. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of GRIR.

#### French Abstract

La presente invention concerne deux recepteurs humains couples a la proteine G associes a une reaction immunitaire (GRIR), ainsi que des polynucleotides identifiant et codant les GRIR. Cette invention concerne par ailleurs des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes. Enfin, cette invention concerne des procedes de traitement et de prevention des troubles associes a l'expression des GRIR.

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DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00643898

**HUMAN CDC10 HOMOLOG**

**HOMOLOGUE CDC10 HUMAIN**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter  
Drive, Palo Alto, CA 94304, US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L., 230 Monroe Drive & 12, Mountain  
View, CA 94040, US

YUE Henry, YUE, Henry, 826 Lois Avenue, Sunnyvale, CA 94087, US

GUEGLER Karl J, GUEGLER, Karl, J., 1048 Oakland Avenue, Menlo Park, CA  
94025, US

KASER Matthew R, KASER, Matthew, R., 4793 Ewing Road, Castro Valley, CA  
94546- 1017, US

MATHUR Preete, MATHUR, Preete, 43733 Greenhills, Fremont, CA 94539, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9927095 A1 19990603

Application: WO 98US24098 19981112 (PCT/WO US9824098)

Priority Application: US 97978182 19971125

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; C07K-014/475; C07K-016/18;

C12N-005/10; A61K-038/17; C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description  
Claims  
Fulltext Word Count: 20232

English Abstract

The invention provides a human growth-related CDC10 homolog (GRSEP) and polynucleotides which identify and encode GR-SEP. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating and preventing disorders associated with expression of GR-SEP.

French Abstract

La presente invention concerne un homologue CDC10 (GR-SEP) associe a la croissance humaine et des polynucleotides qui identifient et codent pour GR-SEP. Cette invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne, en outre, des procedes de traitement et de prevention des troubles associes a une expression de GR-SEP.

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DIALOG(R) File 349:PCT Fulltext  
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00643883 \*\*Image available\*\*

**HUMAN PROTEIN KINASE AND KINASE INHIBITORS**  
**PROTEINE KINASE HUMAINE ET INHIBITEURS DE KINASE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US

BANDMAN Olga, BANDMAN, Olga , 366 Anna Avenue, Mountain View, CA 94043 ,  
US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9927080 A2 19990603

Application: WO 98US24100 19981112 (PCT/WO US9824100)

Priority Application: US 97977816 19971125

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-009/12;

International Patent Class: C12N-015/54; C12N-005/10; C12N-001/21;

A61K-038/45; C07K-016/40; C12P-021/02; C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23923

English Abstract

The invention provides a human protein kinase (PK) and kinase inhibitors (PKI) and polynucleotides which identify and encode PK and PKI. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of PK and PKI.

French Abstract

L'invention concerne une proteine kinase humaine (PK), des inhibiteurs de kinase (PKI), et des polynucleotides qui identifient et codent pour PK et PKI. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne, en outre, des procedes de diagnostic, de traitement ou de prevention des troubles associes a une expression de PK et de PKI.

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DIALOG(R) File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00641469 \*\*Image available\*\*

HUMAN PHOSPHOLIPASE A2 PROTEIN AND DNA ENCODING IT

PROTEINE DE TYPE PHOSPHOLIPASE A2 D'ORIGINE HUMAINE ET ADN LA CODANT

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HAWKINS Phillip R, HAWKINS, Phillip, R. , 750 N. Shoreline Boulevard &96, Mountain View, CA 94034 , US

BANDMAN Olga, BANDMAN, Olga , 366 Anna Avenue, Mountain View, CA 94043 , US

GUEGLER Karl J, GUEGLER, Karl, J. , 1048 Oakland Avenue, Menlo Park, CA 94025 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9924587 A2 19990520

Application: WO 98US23555 19981104 (PCT/WO US9823555)

Priority Application: US 97966317 19971107

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/55;

International Patent Class: C12N-009/20; C12N-015/70; C12N-005/10;

C12P-021/00; A61K-038/46; C12Q-001/68; C07K-016/40;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19071

English Abstract

The invention provides a human phospholipase A2 protein PHPLA2 and polynucleotides which identify and encode PHPLA2. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of PHPLA2.

French Abstract

La presente invention concerne une proteine de type phospholipase A2 d'origine humaine, la PHPLA2 et des polynucleotides qui identifient et code la PHPLA2. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps, et des antagonistes. L'invention concerne enfin des traitements concernant des troubles lies a l'expression de la PHPLA2.

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DIALOG(R) File 349:PCT Fulltext

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00640244    \*\*Image available\*\*

**SUPPRESSOR OF CYTOKINE SIGNALING**

**SUPPRESSEUR DE LA SIGNALISATION CYTOKINAIRE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US  
SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US  
CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9923220 A1 19990514

Application: WO 98US22930 19981028 (PCT/WO US9822930)

Priority Application: US 97963165 19971103

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; C12N-015/70; C12N-001/21;

A61K-038/17; C07K-016/18; C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19198

**English Abstract**

The invention provides a human suppressor of cytokine signaling (HSCS-1)  
and polynucleotides which identify and encode HSCS-1. The invention also  
provides expression vectors, host cells, agonists, antibodies and  
antagonists. The invention also provides methods for treating disorders  
associated with expression of HSCS-1.

**French Abstract**

La presente invention concerne un suppresneur humain de la signalisation  
cytokinaire (HSCS-1) et des polynucleotides identifiant et codant le  
HSCS-1. L'invention concerne egalement des vecteurs d'expression, des  
cellules hotes, des agonistes, des anticorps, et des antagonistes.  
L'invention concerne enfin le traitement de troubles associes a  
l'expression du HSCS-1.

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DIALOG(R)File 349:PCT Fulltext

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00638925    \*\*Image available\*\*

**HUMAN VESICLE MEMBRANE PROTEIN-LIKE PROTEINS**

**PROTEINES DE TYPE PROTEINE DE MEMBRANE DE VESICULE HUMAINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US  
YUE Henry, YUE, Henry , 826 Lois Avenue, Sunnyvale, CA 94087 , US  
CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US  
LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9921994 A2 19990506  
Application: WO 98US21730 19981014 (PCT/WO US9821730)  
Priority Application: US 97959004 19971028  
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG  
Main International Patent Class: C12N-015/12;  
International Patent Class: C07K-014/47; A61K-038/17; C12N-015/11;  
A61K-031/70; C07K-016/18; A61K-039/395; C12Q-001/68; G01N-033/68;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 24126

#### English Abstract

The invention provides three human vesicle membrane protein-like proteins (VMP) and polynucleotides which identify and encode VMP. The invention also provides expression vectors, host cells, agonists, antibodies, and antagonists. The invention also provides methods for treating and preventing disorders associated with expression of VMP.

#### French Abstract

L'invention concerne trois proteines de type proteines de membrane de vesicule humaine (VMP) et des polynucleotides qui identifient et codent ces VMP. La presente invention concerne egalement des vecteurs, des cellules hotes, des agonistes, des anticorps et des antagonistes. Elle concerne encore des procedes de traitement et de prevention de troubles associes a l'expression des VMP.

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DIALOG(R) File 349:PCT Fulltext  
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00636398 \*\*Image available\*\*

#### MAMMOGLOBIN HOMOLOG

#### HOMOLOGUE DE LA MAMMOGLOBINE

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US

MURRY Lynn E, MURRY, Lynn, E. , 1124 Los Trancos Road, Portola Valley, CA  
94028 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9919487 A1 19990422

Application: WO 98US21729 19981014 (PCT/WO US9821729)

Priority Application: US 97951750 19971016

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; A61K-038/17; G01N-033/53;

C12Q-001/68; C12N-015/11; A61K-031/70; C07K-016/18; A61K-039/395;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims  
Fulltext Word Count: 18940

English Abstract

The invention provides a human mammoglobin homolog (HMH) and polynucleotides which identify and encode HMH. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of HMH.

French Abstract

L'invention porte sur un homologue de la mammoglobine humaine (HMH), sur des polynucleotides l'identifiant et codant pour elle, sur des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps, et des antagonistes. Elle porte egalement sur des procedes permettant de diagnostiquer, traiter, ou prevenir des troubles associes a l'expression de la HMH.

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DIALOG(R) File 349:PCT Fulltext  
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00636397

CELL DIVISION REGULATORS  
REGULATEURS DE LA DIVISION CELLULAIRE

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive #12, Mountain View, CA 94040 , US

BANDMAN Olga, BANDMAN, Olga , 366 Anna Avenue, Mountain View, CA 94043 , US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive #5, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue #30, Mountain View, CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9919486 A1 19990422

Application: WO 98US21728 19981014 (PCT/WO US9821728)

Priority Application: US 97951148 19971015

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; A61K-038/17; C07K-016/18;

G01N-033/566; C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23027

English Abstract

The invention provides three human cell division regulators (HCDR) and polynucleotides which identify and encode HCDR. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for preventing and treating disorders associated with expression of HCDR.

French Abstract

L'invention porte sur trois regulateurs de la division de la cellule humaine (HCDR), sur des polynucleotides l'identifiant et codant pour eux, sur des vecteurs d'expression, des cellules hotes, des agonistes, des



anticorps, et des antagonistes. Elle porte également sur des procedes permettant de diagnostiquer, traiter, ou prevenir des troubles associes a l'expression des HCDR.

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DIALOG(R)File 349:PCT Fulltext

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00636394

**HUMAN PROGESTERONE RECEPTOR COMPLEX P23-LIKE PROTEIN**

**PROTEINE DE TYPE P23 DE COMPLEXE DE RECEPTEUR DE PROGESTERONE HUMAIN**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

YUE Henry, YUE, Henry , 826 Lois Avenue, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue #30, Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive #5, Sunnyvale, CA 94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9919483 A1 19990422

Application: WO 98US21402 19981009 (PCT/WO US9821402)

Priority Application: US 97948197 19971009

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C12N-005/10; C12N-001/21; C07K-014/47; C07K-016/18; C12Q-001/68; G01N-033/50; A61K-038/17;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19771

**English Abstract**

The invention provides a human progesterone receptor complex p23- like protein (PR23P) and polynucleotides which identify and encode PR23P. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating and preventing disorders associated with expression of PR23P.

**French Abstract**

L'invention concerne une proteine de type p23 de complexe de recepteur de progesterone humain (PR23P) et des polynucleotides identifiant et codant PR23P. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne enfin des methodes de traitement et de prevention de troubles associes a l'expression de PR23P.

31/5/15

DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00636393

\*\*Image available\*\*

**VESICLE TRANSPORT ASSOCIATED PROTEINS**

**PROTEINES ASSOCIEES AU TRANSPORT DES VESICULES**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive #12,

Mountain View, CA 94040 , US  
LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US  
CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9919482 A2 19990422  
Application: WO 98US21314 19981009 (PCT/WO US9821314)  
Priority Application: US 97948616 19971010

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/705; C07K-014/47; A61K-038/17;

C12Q-001/68; C07K-016/28; C07K-016/18;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23303

English Abstract

The invention provides human vesicle transport associated proteins (VTAP) and polynucleotides which identify and encode VTAP. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of VTAP.

French Abstract

L'invention concerne des proteines humaines associees au transport des vesicules et des polynucleotides identifiant et codant ces proteines. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne en outre des procedes permettant de traiter les desordres associes a l'expression des proteines considerees.

31/5/16

DIALOG(R) File 349:PCT Fulltext

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00632585 \*\*Image available\*\*

**CELL GROWTH REGULATOR**

**REGULATEUR DE LA CROISSANCE CELLULAIRE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US  
SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9915661 A1 19990401  
Application: WO 98US20001 19980922 (PCT/WO US9820001)  
Priority Application: US 97934845 19970922

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; C07K-016/18; A61K-038/17;  
C12Q-001/68; G01N-033/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19384

#### English Abstract

The invention provides a human cell growth regulator (CELR) and polynucleotides which identify and encode CELR. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of CELR.

#### French Abstract

L'invention concerne un regulateur de la croissance cellulaire humaine (CELR) et des polynucleotides qui identifient et codent CELR. Elle porte aussi sur des vecteurs d'expression, des cellules hotes, des agonistes et des antagonistes, ainsi que sur des methodes de traitement de troubles associes a l'expression de CELR.

31/5/17

DIALOG(R) File 349: PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00632583 \*\*Image available\*\*

**HUMAN UBIQUITIN-CONJUGATING ENZYMES**

**ENZYMES DE CONJUGAISON DE L'UBIQUITINE HUMAINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive & 12, Mountain View, CA 94040 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue & 30, Mountain View, CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9915659 A2 19990401

Application: WO 98US19970 19980922 (PCT/WO US9819970)

Priority Application: US 97933750 19970923; US 97965689 19971106

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES

FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; A61K-038/17; C12N-015/63;

C07K-016/18; C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20372

#### English Abstract

The invention provides a human ubiquitin-conjugating enzyme (HUBI) and polynucleotides which identify and encode HUBI. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of HUBI.

#### French Abstract

L'invention concerne une enzyme de conjugaison de l'ubiquitine humaine

(HUBI) et des polynucleotides qui identifient et codent HUBI. L'invention porte également sur des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention se rapporte encore a des procedes de diagnostic, de traitement et de prevention de troubles associes a l'expression d'HUBI.

31/5/18

DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00632509 \*\*Image available\*\*

**RAS-LIKE PROTEIN**

**PROTEINE DE TYPE RAS**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive #12, Mountain View, CA 94040 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue #30, Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive #5, Sunnyvale, CA 94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9915554 A2 19990401

Application: WO 98US19443 19980917 (PCT/WO US9819443)

Priority Application: US 97935333 19970922

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C07K-014/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19603

**English Abstract**

The invention provides a human Ras-like protein (PRAS) and polynucleotides which identify and encode PRAS. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of PRAS.

**French Abstract**

L'invention concerne une proteine de type RAS (PRAS) humaine et des polynucleotides qui identifient et codent pour PRAS. L'invention concerne également des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne également des methodes de traitement de troubles associes a l'expression de PRAS.

31/5/19

DIALOG(R)File 349:PCT Fulltext

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00631264

**HUMAN PRL-1 PHOSPHATASE**

**PHOSPHATASE PRL-1 HUMAINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

AU-YOUNG Janice, AU-YOUNG, Janice , 1419 Kains Avenue, Berkeley, CA 94702

, US

GUEGLER Karl J, GUEGLER, Karl, J. , 1048 Oakland Avenue, Menlo Park, CA  
94025 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9914340 A1 19990325

Application: WO 98US19805 19980921 (PCT/WO US9819805)

Priority Application: US 97934169 19970919

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/55;

International Patent Class: C12N-009/16; A61K-038/46; C12Q-001/68;  
C12N-015/11; C07K-016/40;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 18833

#### English Abstract

The invention provides a human PRL-1 phosphatase (HPRL-1) and polynucleotides which indentify and encode HPRL-1. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of HPRL-1.

#### French Abstract

Cette invention concerne une phosphatase PRL-1 humaine (HPRL-1) et des polynucleotides qui identifient et codent HPRL-1; des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes; ainsi que des procedes permettant de traiter des pathologies associees a l'expression de HPRL-1.

31/5/20

DIALOG(R) File 349:PCT Fulltext

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00631254 \*\*Image available\*\*

**HUMAN MACROPHAGE RECEPTOR MARCO**

**RECEPTEUR MACROPHAGE HUMAIN DE TYPE MARCO**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

BANDMAN Olga, BANDMAN, Olga , 366 Anna Avenue, Mountain View, CA 94043 ,  
US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9914329 A1 19990325

Application: WO 98US19551 19980917 (PCT/WO US9819551)

Priority Application: US 97934168 19970919

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C12N-015/11; C12N-015/70; C12N-001/21;  
C12Q-001/68; C07K-014/705; C07K-016/28; A61K-038/17; A61K-039/395;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 19074

#### English Abstract

The invention provides a human MARCO (MCCOL) and polynucleotides which identify and encode MCCOL. The invention also provides expression vectors, host cells, agonists, antibodies, and antagonists. The invention also provides methods for treating and preventing disorders associated with expression of MCCOL.

#### French Abstract

La presente invention concerne un recepteur macrophage humain de type MARCO (MCCOL) et des polynucleotides qui identifient et codent MCCOL. La presente invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. La presente invention concerne, en outre, des procedes de traitement et de prevention des troubles associes a l'expression de MCCOL.

31/5/21

DIALOG(R) File 349:PCT Fulltext  
(c) 2001 WIPO/MicroPat. All rts. reserv.

00629983 \*\*Image available\*\*

#### CXC CHEMOKINE CHIMIOKINE CXC

#### Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

#### Inventor(s):

NEOTE Kuldeep, NEOTE, Kuldeep , 15 Rose Lane, East Lyme, CT 06333 , US  
STRICK Christine A, STRICK, Christine, A. , 72 Bushnell Road, Lisbon, CT  
06351 , US  
SAHAGAN Barbara G, SAHAGAN, Barbara, G. , 3 Griffen Drive, Mystic, CT  
06355 , US  
MURRY Lynn E, MURRY, Lynn, E. , 1124 Los Trancos Road, Portola Valley, CA  
94028 , US

#### Patent and Priority Information (Country, Number, Date):

Patent: WO 9913082 A1 19990318  
Application: WO 98US18940 19980911 (PCT/WO US9818940)  
Priority Application: US 97928954 19970912

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/19;  
International Patent Class: C07K-014/52; A61K-038/19; A61K-039/395;  
A61K-031/70; C12Q-001/68; G01N-033/68; C07K-016/24;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 20586

#### English Abstract

The invention provides a human CXC chemokine (I-TAC) and polynucleotides which identify and encode I-TAC. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of I-TAC.

French Abstract

L'invention concerne une chimiokine humaine CXCL12 (I-TAC) et des polynucleotides qui identifient et codent cette I-TAC. L'invention concerne également des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne en outre des procedes relatifs au traitement des troubles associes a l'expression de ladite I-TAC.

31/5/22

DIALOG(R)File 349:PCT Fulltext

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00629961

**HUMAN SIGMA RECEPTOR**

**RECEPTEUR SIGMA HUMAIN**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12, Mountain View, CA 94040 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte &5, Sunnyvale, CA 94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9913055 A1 19990318

Application: WO 98US18941 19980911 (PCT/WO US9818941)

Priority Application: US 97928612 19970912

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12N-005/12;

International Patent Class: C12N-005/10; C07K-014/705; C07K-016/28; C12Q-001/68; A61K-038/17;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19242

English Abstract

The invention provides a human sigma receptor (SIGR) and polynucleotides which identify and encode SIGR. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of SIGR.

French Abstract

L'invention concerne un recepteur sigma humain (SIGR) et des polynucleotides identifiant et codant ce SIGR. L'invention concerne également des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne en outre des procedes qui permettent de traiter les troubles associes a l'expression du SIGR.

31/5/23

DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00628724

NEW VRK1 KINASE

**NOUVELLE KINASE VRK1**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter Drive, Palo Alto, CA 94304, US

Inventor(s):

YUE Henry, YUE, Henry, 826 Lois Avenue, Sunnyvale, CA 94087, US

LAL Preeti, LAL, Preeti, 2382 Lass Drive, Santa Clara, CA 95054, US

CORLEY Neil C, CORLEY, Neil, C., 1240 Dale Avenue &30, Mountain View, CA 94040, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9911794 A2 19990311

Application: WO 98US18524 19980904 (PCT/WO US9818524)

Priority Application: US 97923469 19970904

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/54;

International Patent Class: C12N-009/12; C12P-021/00; A61K-038/45; C12Q-001/68; C07K-016/40;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 18918

**English Abstract**

The invention provides a new VRK1 kinase (NVRK1) and polynucleotides which identify and encode NVRK1. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of NVRK1.

**French Abstract**

L'invention concerne une nouvelle kinase VRK1 (NVRK1) et des polynucleotides identifiant et codant la NVRK1. L'invention concerne également des vecteurs d'expression, des cellules hftes, des agonistes, des anticorps et des antagonistes. L'invention concerne, en outre, des methodes de traitement de troubles associes C l'expression de la NVRK1.

31/5/24

DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00628723 \*\*Image available\*\*

**HUMAN < i> act < /i> VA-ORF4-LIKE PROTEIN**

**PROTEINE HUMAINE DE TYPE < i> act < /i> VA-ORF4**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter Drive, Palo Alto, CA 94304, US

Inventor(s):

LAL Preeti, LAL, Preeti, 2382 Lass Drive, Santa Clara, CA 95054, US

TANG Tom Y, TANG, Tom, Y., 4230 Ranwick Court, San Jose, CA 95118, US

CORLEY Neil C, CORLEY, Neil, C., 1240 Dale Avenue &30, Mountain View, CA 94040, US

SHAH Purvi, SHAH, Purvi, 1608 Queen Charlotte Drive &5, Sunnyvale, CA 94087, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9911793 A1 19990311

Application: WO 98US18395 19980903 (PCT/WO US9818395)

Priority Application: US 97923856 19970903

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE



CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/53;

International Patent Class: C12N-009/04; C12N-015/70; C12N-001/21;

A61K-038/44; C07K-016/40; C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19727

#### English Abstract

The invention provides a human < i> act < /i> VA-ORF4-like protein (A-ORFP) and polynucleotides which identify and encode A-ORFP. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of A-ORFP.

#### French Abstract

L'invention concerne une proteine humaine de type < i> act < /i> VA- ORF4 (A-ORFP) et des polynucleotides identifiant et codant A-ORFP. L'invention concerne egalement des vecteurs d'expression, des cellules hftes, des agonistes, des anticorps et des antagonistes. L'invention concerne aussi des methodes de traitement des troubles associes C l'expression de A-ORFP.

31/5/25

DIALOG(R) File 349:PCT Fulltext

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00627408

**HUMAN NUCLEOTIDE PYROPHOSPHOHYDROLASE**

**NUCLEOTIDE PYROPHOSPHOHYDROLASE HUMAIN**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

MITCHELL Peter G, MITCHELL, Peter, G. , 5 Godfrey Street, Mystic, CT  
06355 , US

HUTCHINSON Nancy, HUTCHINSON, Nancy , 7 Squire Hill, Old Lyme, CT 06371 ,  
US

LAWTON Michael, LAWTON, Michael , 61 Magna Lane, Westbrook, CT 06498 , US  
MAGNA Holly, MAGNA, Holly , 88 Old Black Point Road, Niantic, CT 06357 ,  
US

YOCUM Sue A, YOCUM, Sue, A. , 11 Pinecrest Lane, Baltic, CT 06330 , US

MURRY Lynn E, MURRY, Lynn, E. , 1124 Los Trancos Road, Portola Valley, CA  
94028 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9910502 A1 19990304

Application: WO 98US17648 19980826 (PCT/WO US9817648)

Priority Application: US 97918914 19970827

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FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/55;

International Patent Class: C12N-009/16; A61K-038/46; C07K-016/40;

C12N-015/11; A61K-031/70; A61K-039/395; C12Q-001/68; G01N-033/53;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19953

English Abstract

The invention provides a human nucleotide pyrophosphohydrolase (NTPPH-1) and polynucleotides which identify and encode NTPPH-1. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of NTPPH-1.

French Abstract

La presente invention concerne un nucleotide pyrophosphohydrolase humain (NTPPH-1) et des polynucleotides qui identifient et codent NTPPH-1. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne, en outre, des procedes de traitement de troubles associes a une expression de NTPPH-1.

31/5/26

DIALOG(R)File 349:PCT Fulltext

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00627398

**NEW HUMAN G-PROTEIN COUPLED RECEPTOR**

**RECEPTEUR COUPLE A LA PROTEINE G HUMAINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

GUEGLER Karl J, GUEGLER, Karl, J. , 1048 Oakland Avenue, Menlo Park, CA 94025 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9910491 A1 19990304

Application: WO 98US17690 19980826 (PCT/WO US9817690)

Priority Application: US 97919624 19970828

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FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C12N-015/70; C12N-001/21; C07K-014/705;

C07K-016/28; C12Q-001/68; A61K-038/17;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19172

English Abstract

The invention provides a human G-protein coupled receptor (GReCH) and polynucleotides which identify and encode GReCH. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of GReCH.

French Abstract

L'invention concerne un recepteur couple a la proteine G humaine (GReCH) et des polynucleotides qui identifient et codent GReCH. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne en outre des procedes qui permettent de traiter les troubles associes a l'expression de GReCH.

31/5/27  
DIALOG(R)File 349:PCT Fulltext  
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00626108 \*\*Image available\*\*

**RAB PROTEINS**  
**PROTEINES RAB**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9909182 A2 19990225

Application: WO 98US16983 19980817 (PCT/WO US9816983)

Priority Application: US 97916901 19970821

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/55;

International Patent Class: C12N-009/16; A61K-038/46; C12Q-001/68;  
C12N-015/70; C12N-001/21;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 23097

**English Abstract**

The invention provides three human Rab proteins (RABP) and polynucleotides which identify and encode RABP. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of RABP.

**French Abstract**

L'invention porte sur trois proteines Rab humaines (RABP) et les polynucleotides qui les identifient et codent pour elles. Elle porte en outre sur des vecteurs d'expression, des cellules hotes, des agonistes, anticorps et antagonistes, et sur des procedes de traitement de troubles associes a l'expression du RABP.

31/5/28

DIALOG(R)File 349:PCT Fulltext  
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00626100

**UBC7-LIKE UBIQUITIN-CONJUGATING ENZYME**

**ENZYME DE CONJUGAISON DE L'UBIQUITINE RESSEMBLANT A UBC7**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue, &30, Mountain View,  
CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9909171 A1 19990225  
Application: WO 98US17320 19980820 (PCT/WO US9817320)  
Priority Application: US 97918723 19970821  
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG  
Main International Patent Class: C12N-015/12;  
International Patent Class: C12N-015/54; C12N-009/12; C12N-009/64;  
C12N-005/10; C12P-021/02; C07K-016/18; C12Q-001/68; A61K-038/17;  
A61K-038/45;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 19739

#### English Abstract

The invention provides a human ubiquitin-conjugating enzyme (HUCE- 1) and polynucleotides which identify and encode HUCE-1. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of HUCE-1.

#### French Abstract

L'invention concerne une enzyme de conjugaison de l'ubiquitine humaine (HUCE-1) et des polynucleotides qui identifient et codent HUCE-1. Elle porte aussi sur des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. Elle se rapporte encore a des methodes de traitement de troubles associes a l'expression de HUCE-1.

31/5/29

DIALOG(R) File 349:PCT Fulltext  
(c) 2001 WIPO/MicroPat. All rts. reserv.

00626099 \*\*Image available\*\*

#### HIGH AFFINITY IMMUNOGLOBULIN E RECEPTOR-LIKE PROTEIN PROTEINE RESSEMBLANT AU RECEPTEUR DE L'IMMUNOGLOBULINE E DE HAUTE AFFINITE

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

BANDMAN Olga, BANDMAN, Olga , 366 Anna Avenue, Mountain View, CA 94043 ,  
US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9909170 A1 19990225  
Application: WO 98US17271 19980820 (PCT/WO US9817271)  
Priority Application: US 97916902 19970821

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;  
International Patent Class: C07K-014/705; C12N-015/70; C12N-001/21;  
A61K-038/17; C07K-016/28; C12Q-001/68;  
Publication Language: English  
Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims

Fulltext Word Count: 19616

English Abstract

The invention provides a human high affinity immunoglobulin E receptor-like protein (IGERB) and polynucleotides which identify and encode IGERB. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of IGERB.

French Abstract

L'invention concerne une proteine ressemblant au recepteur de l'immunoglobuline E de haute affinite (IGERB) et des polynucleotides qui identifient et codent IGERB. Elle porte encore sur des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. Elle porte aussi sur des methodes de traitement de troubles associes a l'expression d'IGERB.

31/5/30

DIALOG(R) File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00624767

**HUMAN RETICULOCALBIN ISOFORMS**

**ISOFORMES DE RETICULOCALBINES HUMAINES**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter Drive, Palo Alto, CA 94304, US

Inventor(s):

BANDMAN Olga, BANDMAN, Olga, 366 Anna Avenue, Mountain View, CA 94043, US  
HILLMAN Jennifer L, HILLMAN, Jennifer, L., 230 Monroe Drive #12, Mountain View, CA 94040, US

LAL Preeti, LAL, Preeti, 2382 Lass Drive, Santa Clara, CA 95054, US

CORLEY Neil C, CORLEY, Neil, C., 1240 Dale Avenue #30, Mountain View, CA 94040, US

SHAH Purvi, SHAH, Purvi, 1608 Queen Charlotte Drive #5, Sunnyvale, CA 94087, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9907849 A2 19990218

Application: WO 98US16259 19980805 (PCT/WO US9816259)

Priority Application: US 97910927 19970808

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; A61K-038/17; G01N-033/68; C12Q-001/68; C07K-016/18;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 22123

English Abstract

The invention provides two human reticulocalbin isoforms designated individually as RCN  $\gamma$ ; and RCN  $\delta$ ; and collectively as RCN, and polynucleotides which identify and encode RCN. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of RCN.

French Abstract

L'invention concerne deux isoformes de reticulocalbines humaines, RCN  $\gamma$ ; et RCN  $\delta$ ;,, denommees collectivement RCN, ainsi que les polynucleotides qui identifient et codent RCN. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes,

des anticorps, et des antagonistes. L'invention concerne enfin des  
procedes de traitement des dysfonctionnements lies a l'expression de RCN.

31/5/31

DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00623456 \*\*Image available\*\*

**SYNAPTOJANIN ISOFORM**

**ISOFORME DE SYNAPTOJANINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

TANG Tom Y, TANG, Tom, Y. , 4230 Ranwick Court, San Jose, CA 95118 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9906572 A1 19990211

Appliation: WO 98US15782 19980728 (PCT/WO US9815782)

Priority Application: US 97904234 19970731

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FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD

MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US

UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE

CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN

GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/55;

International Patent Class: C12N-009/16; C07K-016/40; C12N-001/21;

C12Q-001/68; A61K-038/46;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19973

**English Abstract**

The invention provides a human synaptojanin isoform (NSYN-1) and polynucleotides which identify and encode NSYN-1. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of NSYN-1.

**French Abstract**

Cette invention concerne une isoforme de synaptojanine humaine (NSYN-1) ainsi que des polynucleotides qui identifient et codent NSYN-1. Cette invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagoniste, ainsi que des procedes qui permettent de traiter des troubles associes a l'expression de NSYN-1.

31/5/32

DIALOG(R)File 349:PCT Fulltext

(c) 2001 WIPO/MicroPat. All rts. reserv.

00623445 \*\*Image available\*\*

**ANNEXIN BINDING PROTEIN**

**PROTEINE DE LIAISON AUX ANNEXINES**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive #12,

Mountain View, CA 94040 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue #30, Mountain View, CA

94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive #5, Sunnyvale, CA

94087 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9906560 A1 19990211

Application: WO 98US15599 19980728 (PCT/WO US9815599)

Priority Application: US 97903801 19970731

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; A61K-038/17; C07K-016/18;

C12N-015/85; C12N-005/10; C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 19302

#### English Abstract

The invention provides a human annexin binding protein (NABP-1) and polynucleotides which identify and encode NABP-1. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of NABP-1.

#### French Abstract

Cette invention se rapporte a une proteine de liaison aux annexines (NABP-1) et a des polynucleotides qui identifient et codent ladite proteine NABP-1. Cette invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. Elle se rapporte en outre a des methodes de traitement des troubles associes a l'expression de la proteine NABP-1.

31/5/33

DIALOG(R) File 349:PCT Fulltext

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00623443

#### HUMAN LONGEVITY-ASSURANCE PROTEIN HOMOLOGS

#### HOMOLOGUES PROTEIQUES D'ASSURANCE DE LONGEVITE HUMAINE

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter  
Drive, Palo Alto, CA 94304, US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L., 230 Monroe Drive &12, Mountain  
View, CA 94040, US

CORLEY Neil C, CORLEY, Neil, C., 1240 Dale Avenue &30, Mountain View, CA  
94040, US

SHAH Purvi, SHAH, Purvi, 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087, US

LAL Preeti, LAL, Preeti, 2382 Lass Drive, Santa Clara, CA 95054, US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9906558 A1 19990211

Application: WO 98US15591 19980728 (PCT/WO US9815591)

Priority Application: US 97902853 19970730

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES  
FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD  
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US  
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE  
CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN  
GW ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C12N-005/10; C12N-001/21; C07K-014/47;

C07K-016/18; C12Q-001/68; G01N-033/50; A61K-038/17;

Publication Language: English

Filing Language: English  
Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 21627

#### English Abstract

The invention provides two human longevity-assurance protein homologs, designated individually as LAPH-1 and LAPH-2 and collectively as LAPH, and polynucleotides which identify and encode LAPH. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of LAPH.

#### French Abstract

Cette invention se rapporte a deux homologues proteiques d'assurance de longevite humaine, designes individuellement par les termes LAPH-1 et LAPH-2 et collectivement par le terme LAPH, ainsi qu'a des polynucleotides qui identifient et codent LAPH. Cette invention se rapporte egalement a des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes associes. Elle concerne en outre des methodes de traitement des troubles associes a l'expression du LAPH.

31/5/34

DIALOG(R) File 349:PCT Fulltext  
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00620521

#### HUMAN MATRILIN-3

#### MATRILINE-3 HUMAINE

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12, Mountain View, CA 94040 , US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA 94087 , US

KASER Matthew R, KASER, Matthew, R. , 4793 Ewing Road, Castro Valley, CA 94546- 1017 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9904002 A1 19990128

Application: WO 98US15092 19980721 (PCT/WO US9815092)

Priority Application: US 97897443 19970721

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FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG

MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ

VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH

CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW

ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/78; A61K-038/17; C07K-016/18;

C12Q-001/68;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20130

#### English Abstract

The invention provides a human matrilin-3 (MAT-3) and polynucleotides which identify and encode MAT-3. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention



also provides methods for treating disorders associated with expression of MAT-3.

French Abstract

L'invention concerne une matriline-3 (MAT-3) humaine et des polynucleotides identifiant et codant MAT-3. Elle concerne également des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention porte enfin sur des methodes de traitement des troubles associes a l'expression de MAT-3.

31/5/35

DIALOG(R) File 349:PCT Fulltext

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00620515 \*\*Image available\*\*

T-CELL RECEPTOR BETA-LIKE PROTEIN

PROTEINE DE TYPE BETA RECEPTEUR DES CELLULES T

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12, Mountain View, CA 94040 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA 94040 , US

Patent and Priority Information (Country, Number, Date):

Patent: WO 9903995 A1 19990128

Application: WO 98US14598 19980717 (PCT/WO US9814598)

Priority Application: US 97897097 19970718

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FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG

MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ

VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH

CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW

ML MR NE SN TD TG

Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/705; C07K-016/18; C12Q-001/68;

A61K-038/17;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20549

English Abstract

The invention provides a human T-cell receptor beta-like protein (TCRLP) and polynucleotides which identify and encode TCRLP. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of TCRLP.

French Abstract

L'invention concerne une proteine humaine de type beta recepteur des cellules T (TCRLP) et des polynucleotides identifiant et codant ladite proteine TCRLP. L'invention concerne également des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. Elle concerne aussi des procedes pour le traitement de troubles lies a l'expression de la proteine TCRLP consideree.

31/5/36

DIALOG(R) File 349:PCT Fulltext

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00620509 \*\*Image available\*\*

HUMAN ATP-BINDING CASSETTE TRANSPORT PROTEIN

PROTEINE HUMAINE DE TRANSPORT DE CASSETTE DE LIAISON DE L'ADENOSINE TRIPHOSPHATE (ATP)

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jenifer L, HILLMAN, Jenifer, L. , 230 Monroe Drive, #12, Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive, #5, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue, #30, Mountain View, CA 94040 , US

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Detailed Description

Claims

Fulltext Word Count: 18921

English Abstract

The invention provides a human ATP-binding cassette transport protein (ABCTxH) and polynucleotides which identify and encode ABCTxH. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of ABCTxH.

French Abstract

L'invention concerne une proteine humaine de transport de cassette de liaison de l'ATP (ABCTxH) et des polynucleotides identifiant et codant ladite proteine ABCTxH. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. Elle concerne aussi des procedes pour le traitement de troubles lies a l'expression de la proteine ABCTxH considerree.

31/5/37

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00619604

**NEW HUMAN GROWTH REGULATOR PROTEIN**

**NOUVELLE PROTEINE REGULATRICE DE CROISSANCE HUMAINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

BANDMAN Olga, BANDMAN, Olga , 366 Anna Avenue, Mountain View, CA 94043 , US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive #5, Sunnyvale, CA 94087 , US

CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue #30, Mountain View, CA 94040 , US

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Publication Language: English  
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Claims  
Fulltext Word Count: 21715

#### English Abstract

The invention provides a new human growth regulator protein (GRREG) and polynucleotides which identify and encode GRREG. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of GRREG.

#### French Abstract

L'invention concerne une nouvelle proteine regulatrice de croissance humaine (GRREG) et des polynucleotides identifiant et codant GRREG. L'invention concerne egalement des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. L'invention concerne en outre des methodes de traitement de troubles associes a l'expression de GRREG.

31/5/38

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00617364 \*\*Image available\*\*

**PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE 5-PHOSPHATASE FROM HUMAN**  
**PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE 5-PHOSPHATASE TIREE D'UN ETRE HUMAIN**  
Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

#### Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US  
LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
CORLEY Neil C, CORLEY, Neil, C. , 1240 Dale Avenue &30, Mountain View, CA  
94040 , US  
SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087 , US

#### Patent and Priority Information (Country, Number, Date):

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Publication Language: English  
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Fulltext Availability:  
Detailed Description  
Claims  
Fulltext Word Count: 22703

#### English Abstract

The invention provides a human phosphatidylinositol 4,5- bisphosphate 5-phosphatase (PBPP) and polynucleotides which identify and encode PBPP. The invention also provides expression vectors, host cells, agonists,

antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of PBPP.

French Abstract

L'invention a trait a une phosphatidylinositol 4,5-bisphosphate 5-phosphatase humaine (PBPP) et a des polynucleotides qui identifient et codent cette PBPP. Cette invention concerne egalement des vecteurs d'expression, cellules hotes, agonistes, anticorps et antagonistes correspondants ainsi que des procedes permettant de traiter des troubles associes a l'expression de la PBPP.

31/5/39

DIALOG(R)File 349:PCT Fulltext

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00612634 \*\*Image available\*\*

**INSULIN RECEPTOR TYROSINE KINASE SUBSTRATE**

**SUBSTRAT DE LA TYROSINE KINASE DU RECEPTEUR DE L'INSULINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive #12, Mountain View, CA 94040 , US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte Drive #5, Sunnyvale, CA 94086 , US

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Priority Application: US 97878563 19970619

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NE SN TD TG

Main International Patent Class: C07K-014/00;

Publication Language: English

Filing Language: English

Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 20885

English Abstract

The invention provides a human insulin receptor tyrosine kinase substrate (IRS-p53h) and polynucleotides which identify and encode IRS- p53h. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of IRS-p53h.

French Abstract

L'invention porte sur un substrat de tyrosine kinase de l'insuline humaine (IRS-p53h) et sur des polynucleotides qui identifient et codent IRS-p53h; sur des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. Cette invention porte egalement sur des procedes de traitement de troubles associes a l'expression d'IRS-p53h.

31/5/40

DIALOG(R)File 349:PCT Fulltext

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00611596 \*\*Image available\*\*

**HUMAN FORMIN BINDING PROTEIN**

**PROTEINE LIANTE HUMAINE APPELEE FORMINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter

Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US

LAL Preeti, LAL, Preeti , 2382 Lass Drive, Santa Clara, CA 95054 , US  
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Main International Patent Class: C12N-015/12;

International Patent Class: C07K-014/47; C07K-016/18; C12Q-001/68;

A61K-038/17; G01N-033/50;

Publication Language: English

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Detailed Description

Claims

Fulltext Word Count: 19955

English Abstract

The invention provides a human formin binding protein (FBPhu) and polynucleotides which identify and encode FBPhu. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of FBPhu.

French Abstract

L'invention concerne une proteine liante humaine appelee formine (FBPhu) et des polynucleotides qui identifient et codent FBPhu. L'invention concerne en outre des vecteurs d'expression, des cellules hotes, des agonistes, des anticorps et des antagonistes. Elle concerne enfin des methodes pour traiter des troubles associes a l'expression de FBPhu.

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00611584

**HUMAN PEROXISOMAL THIOESTERASE**

**THIOESTERASE PEROXYSOMALE HUMAINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC., 3174 Porter  
Drive, Palo Alto, CA 94304, US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L., 230 Monroe Drive &12, Mountain  
View, CA 94040, US

SHAH Purvi, SHAH, Purvi, 1608 Queen Charlotte Drive &5, Sunnyvale, CA  
94087, US

CORLEY Neil C, CORLEY, Neil, C., 1240 Dale Avenue &30, Mountain View, CA  
94040, US

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Detailed Description

Claims

Fulltext Word Count: 19057

English Abstract  
NotAvailable

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00605212

**HUMAN LYSOPHOSPHOLIPASE**

**LYSOPHOSPHOLIPASE HUMAINE**

Patent Applicant/Assignee:

INCYTE PHARMACEUTICALS INC, INCYTE PHARMACEUTICALS, INC. , 3174 Porter  
Drive, Palo Alto, CA 94304 , US

Inventor(s):

HILLMAN Jennifer L, HILLMAN, Jennifer, L. , 230 Monroe Drive &12,  
Mountain View, CA 94040 , US

SHAH Purvi, SHAH, Purvi , 1608 Queen Charlotte &5, Sunnyvale, CA 94087 ,  
US

MURRY Lynn E, MURRY, Lynn, E. , 1124 Los Trancos Road, Portola Valley, CA  
94028 , US

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Fulltext Availability:

Detailed Description

Claims

Fulltext Word Count: 21379

English Abstract

The invention provides a human lysophospholipase (NHLPL) and polynucleotides which identify and encode NHLPL. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of NHLPL.

French Abstract

Lysophospholipase humaine (NHLPL) et polynucleotides qui identifient et codent ladite NHLPL. La presente invention concerne egalement des vecteurs d'expression, des cellules hotes, des anticorps, des agonistes et des antagonistes, ainsi que des methodes de traitement ou de prophylaxie de troubles associes a l'expression de la NHLPL.

31/5/43

DIALOG(R)File 349:PCT Fulltext

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00404072

**METHOD AND APPARATUS FOR FABRICATING MICROARRAYS OF BIOLOGICAL SAMPLES**

**PROCEDE ET APPAREIL POUR FABRIQUER DES MICROENSEMBLES D'ECHANTILLONS BIOLOGIQUES**

Patent Applicant/Assignee:

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY

Inventor(s):

SHALON Tidhar Dari

BROWN Patrick O

Patent and Priority Information (Country, Number, Date):

Patent: WO 9535505 A1 19951228  
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International Patent Class: G01N-033/68;  
Publication Language: English  
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Claims  
Fulltext Word Count: 11532

English Abstract

A method and apparatus for forming microarrays of biological samples on a support are disclosed. The method involves dispensing a known volume of a reagent at each of a selected array position, by tapping a capillary dispenser on the support under conditions effective to draw a defined volume of liquid onto the support. The apparatus is designed to produce a microarray of such regions in an automated fashion.

Japanese Abstract

L'invention concerne un procede et un appareil pour former des microensembles d'echantillons biologiques sur un support. Le procede consiste a distribuer un volume connu d'un reactif au niveau de chaque position selectionnee de l'ensemble, en ouvrant un distributeur capillaire sur le support dans des conditions permettant d'amener un volume defini de liquide sur le support. L'appareil est concu pour produire automatiquement un microensemble de zones de ce type.

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\$328.31 Estimated cost File349  
\$5.40 TELNET  
\$333.71 Estimated cost this search  
\$333.98 Estimated total session cost 5.825 DialUnits

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## Protein arrays and microarrays

### Heng Zhu\* and Michael Snyder\*†

In the past, studies of protein activities have focused on studying a single protein at a time, which is often time-consuming and expensive. Recently, with the sequencing of entire genomes, large-scale proteome analysis has begun. Arrays of proteins have been used for the determination of subcellular localization, analysis of protein-protein interactions and biochemical analysis of protein function. New protein-microarray technologies have been introduced that enable the high-throughput analysis of protein activities. These have the potential to revolutionize the analysis of entire proteomes.

#### Addresses

\*Department of Molecular, Cellular, and Developmental Biology, and  
†Department of Molecular Biophysics and Biochemistry, Yale University,  
New Haven, Connecticut 06520, USA; e-mail: michael.snyder@yale.edu

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#### Abbreviations

BSA bovine serum albumin  
GST glutathione-S-transferase  
ORF open reading frame

#### Introduction

Large-scale genome sequencing projects have rapidly accelerated the pace of gene discovery and led to the identification of thousands of new genes. The challenge ahead is to identify the functions of the many different genes that encode the organism. Toward this end, a number of new methods have been developed to systematically analyze gene function. One powerful technology that has emerged recently is DNA microarrays. These have allowed the large-scale analysis of gene expression and variations in gene sequence [1–3,4\*\*], and recently, DNA chips coupled with chromatin immunoprecipitation have been used to determine the entire complement of binding sites for transcription factors [5\*\*]. Other genome projects include large-scale gene disruption studies that, although still in their infancy, hold promise for contributing considerable information about gene function through the analysis of mutant phenotypes [4\*\*,6\*\*,7–12].

In most cases, however, a gene function is manifested by the direct activity of its translated protein and, therefore, analysis of protein function is likely to provide a superior approach for elucidating gene function. Consistent with this view, protein levels often do not correlate with mRNA levels [13]. Below, we summarize several recent approaches for the systematic analysis of proteins using protein arrays and microarrays.

#### Large-scale analysis of protein function

Thus far, only a limited number of studies have been performed to analyze protein function on a large scale. Most of

these have been carried out using the budding yeast *Saccharomyces cerevisiae*, the genome of which has been sequenced and encodes 6200 genes [14]. An important part of the work is to generate defined arrays of tagged proteins that allow high-throughput systematic analysis [15]. Subcellular localization of yeast proteins has been carried out using a transposon-tagging strategy to randomly insert hemagglutinin epitope tags into yeast proteins [6\*\*,16]. The tagged strains were assembled into 96-well arrays and the localization patterns of the epitope-tagged proteins determined by indirect immunofluorescence [6\*\*,16]. Using this procedure, approximately 50% of the predicted proteins of yeast have been tagged and many of these have been localized. In addition, potential protein-protein interactions have been identified by two-hybrid approaches [17,18\*\*,19]. In one very impressive study, an array of approximately 5300 yeast proteins individually fused to transcriptional-activation domains were systematically probed with yeast proteins fused to a DNA-binding domain; in this manner, 957 potential protein-protein interactions were revealed [18\*\*].

The ability to generate large numbers of expression clones has allowed the large-scale biochemical analysis of protein function. In a pioneering study, Phizicky and co-workers [20\*\*] used a recombination-cloning strategy to fuse 85–90% of yeast open reading frames (ORFs) to sequences coding glutathione-S-transferase (GST) under the control of a *CUP1* promoter. Yeast strains containing these clones were grouped into around 64 pools of 96 clones; yeast proteins fused to GST were purified from the pools and screened for biochemical activities. Individual strains from positive pools were then screened to find the appropriate yeast strain expressing the activity of interest. Using this method, genes for a number of biochemical activities relating to tRNA splicing (e.g. those for tRNA ligase, 2'-phosphotransferase, phosphodiesterase, cytochrome *c* methyltransferase, and Appr-1'-P-processing activities) were identified. One important advantage of the pooling strategy is that a large number of samples can be rapidly analyzed. Disadvantages are that multiple steps to isolate particular activities are required, and prevalent activities, such as protein kinase activities, must be screened in small pools. In addition, it is difficult to determine if an individual sample that does not give a positive signal has been properly analyzed (i.e. false negatives are difficult to access).

Several methods have also been recently developed for directly screening biochemical activities on a large scale using two-dimensional arrays. Bussow *et al.* [21] prepared a set of 37,830 *Escherichia coli* strains that express His6-tagged fusions from a fetal human brain cDNA library. Fusion proteins were prepared in a 96-well format, spotted onto a filter, and screened for glyceraldehyde-3-phosphate dehydrogenase

activity [22]. The activity of a positive control (a human glyceraldehyde-3-phosphate dehydrogenase) and that from three additional clones were detected; however, the sequences from two of the clones did not match any human proteins while the third represents a short out-of-frame fragment. Thus, further work is needed to examine the quality of the authors' library and/or screening methods.

In an independent approach, Ge [23] used low-density filters to study specific interactions of proteins with DNA, RNA, ligands, and other small chemicals. A filter harboring 48 purified human proteins primarily involved in transcription was prepared and incubated with one of the following: a  $^{32}\text{P}$ -labeled human protein p52; an end-labeled promoter region of an adenovirus; an SV40 early pre-mRNA probe; or a ligand probe. A wide variety of different protein-protein, protein-DNA, protein-RNA, and protein-ligand interactions were detected. Importantly, the protein filters could be reused by stripping the probes with 1 M ammonium sulfate and 1 M urea.

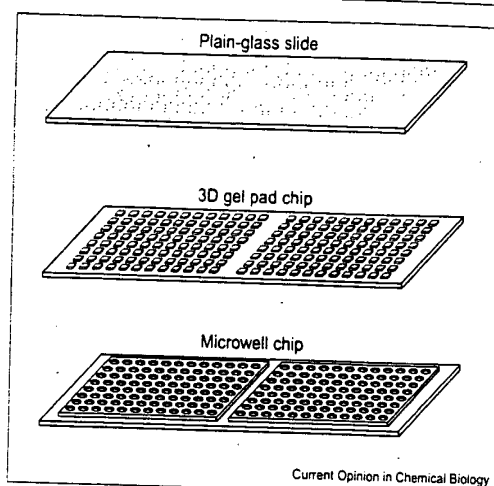
These different approaches have demonstrated that it is possible to prepare large numbers of protein arrays and screen them for biochemical activities, protein-protein interactions and subcellular localization. Although very useful, one significant drawback of the *in vitro* approaches is that they often require large amounts of material. The handling and processing of large numbers of samples is also a concern. Thus, as with the case of DNA chips, procedures that can readily produce and analyze large numbers of samples would greatly facilitate the analysis of large numbers of protein samples [24,25].

### Protein chips

Recently, several groups have developed protein microarray methods for the high-throughput analysis of proteins. Protein microarrays are grids that contain small amounts of purified proteins in a high density. The proteins can be screened in a high-throughput fashion for biochemical activity, protein-protein, protein-DNA, protein-RNA, and protein-ligand interactions.

Several types of chips have been designed; these include glass slides, porous gel pad slides and microwells (Figure 1). Glass slides have the advantage that they can be used with standard microarrays and scanners used for DNA chips. MacBeath and Schreiber [26\*\*] attached proteins to a glass surface activated with a crosslinking agent that reacts with primary amines. Proteins were spotted in a 40% glycerol solution to prevent dehydration. To show that their protein microarrays are feasible for biochemical assays, they tested three known protein-protein interactions, three known kinase-substrate reactions, and three known protein-ligand-binding reactions using fluorophore-tagged proteins, radiolabeled ATP, and synthetic ligands coupled to fluorescently labeled bovine serum albumin (BSA), respectively. In most cases, a small number of arrayed proteins were used, although in one experiment

Figure 1



Three different types of protein microarrays: plain-glass slide, three-dimensional gel pad chip, and microwell chip.

they could identify a single spot of the FKBP12-rapamycin binding protein (FRB) within an array of 10,000 spots containing one other protein. Their work demonstrates the potential of using protein microarrays to perform large-scale protein interaction studies, biochemical assays and analysis of drug targets; however, their study analyzed very few proteins and novel activities were not identified.

Ward and co-workers [27\*\*] also used glass slides for detecting several protein antigens (e.g. human IgE, human prostate-specific Ag). They spotted analytes onto a treated glass surface at high density using either a hand-spotting device or a pin-tool-type microarray robot. The spotted analytes were detected using antibodies attached to an oligonucleotide primer and a clever rolling circle amplification reaction. Their results indicate that this technique has high sensitivity, a wide dynamic range, and excellent spot-to-spot reproducibility.

Instead of spotting proteins onto a two-dimensional solid surface, Mirzabekov and co-workers [28\*\*] described a three-dimensional technique for creating arrays of proteins immobilized inside tiny polyacrylamide gel packets dotted across a glass surface. On a silane-treated glass-slide surface, 3% acrylamide gel is poured with a modified crosslinker —  $N,N'$ -(1,2-dihydroxyethylene) bisacrylamide (DHEBA)-Bis mixture — to improve porosity of the gel. The polymerization is promoted by exposing the acrylamide to a UV source through a mask containing  $100 \times 100 \mu\text{m}$  square windows. The unpolymerized monomers are removed by washing with water. Using this method, they prepared arrays of  $100 \times 100 \times 20 \mu\text{m}$  gel pads spaced  $200 \mu\text{m}$  apart. Proteins or antibodies can be immobilized in the gel pads by either activating the gel with glutaraldehyde or by using a reactive

Table 1

## Features of different types of protein chips.

Glass slides	Matrix slides	Nanowells
Compatible with standard microarrayer and detection equipment	Compatible with standard microarrayer and detection equipment	Compatible with standard microarrayer and detection equipment but requires alignment
High evaporation	Reduce evaporation	Reduce evaporation
Poor for multiple-based reactions	Solution-based reaction can be carried out, but requires longer washing times for material	Versatile for solution-based assays; multiple-component reactions
Inexpensive	Expensive; requires photo lithography to make matrix	Inexpensive
Possible cross-contamination	No cross-contamination	No cross-contamination

hydrazide group that partially substitutes the amide group of the polyacrylamide gel; this allows the crosslinking to oxidized aldehyde groups in the polysaccharide component of the antibodies. Using such technology, Mirzabekov and co-workers [28\*\*] have tested the applications of different types of immunoassays and antigen detection. They have also demonstrated the detection of enzymatic activities for three enzymes immobilized in the gel — horseradish peroxidase, calf intestinal phosphatase and  $\beta$ -D-glucuronidase. Because of the use of three-dimensional gel support, their approach has some important advantages (Table 1). Firstly, the capacity of protein immobilization in the gel pads is much higher than on a two-dimensional glass or plastic surface. Secondly, the homogeneous water environment in the gel reduces drying and thereby minimizes denaturation of proteins. Thirdly, the immobilized proteins are well separated and do not interact with one another. The disadvantages of the gel pads are, firstly, that it is more difficult to change buffers and to recover trapped molecules and, secondly, that it is also more cumbersome to prepare the slides.

Zhu *et al.* [29\*\*] have used both glass slides and a novel nanowell technology for the analysis of yeast protein kinases. 121 of 122 yeast protein kinase genes were cloned into a high-copy expression vector that produces GST fusion proteins under the control of a galactose-inducible promoter, and were purified using a 96-well format (Figure 2; [29\*\*]; H Zhu, S Lee, M Snyder, unpublished data). The samples were then analyzed in a microarray composed of microwells in silicon elastomer sheets.

To demonstrate that this technology is feasible for high-throughput biochemistry assays, kinase assays were performed on 119 protein kinases using 17 different substrates (Figure 2b). The substrates were first bound to the chip through an amino crosslinker, followed by blocking the microwells with BSA. The yeast kinases were then added to each microwell on the chip with radiolabeled  $^{32}\text{P}$ - $\gamma$ -ATP. The chips were washed and then exposed to a phosphorimager to detect the phosphorylated substrate. In an autophosphorylation experiment, in which the kinases phosphorylate themselves, the kinases were first bound to the surface and then incubated in the presence of  $^{32}\text{P}$ - $\gamma$ -ATP. Because over 60% of the kinase proteins showed activities in an

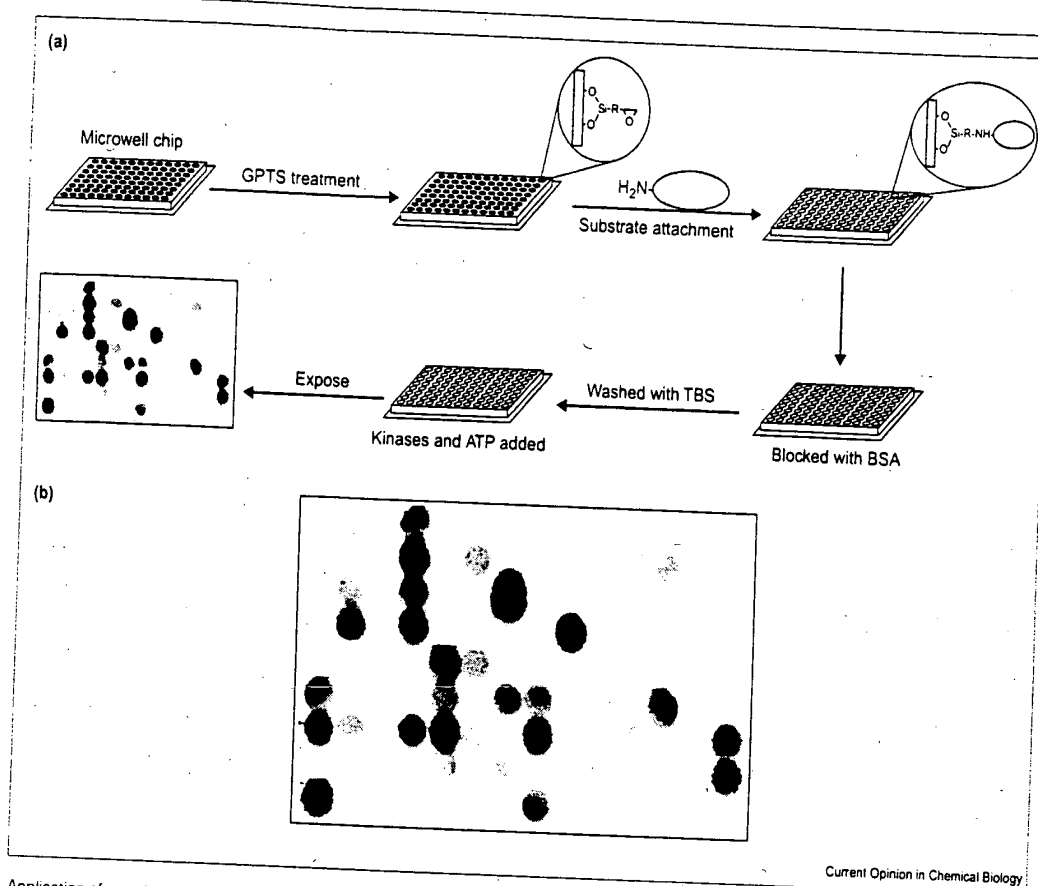
autophosphorylation experiment, the crosslinking of proteins to the chips does not interfere with enzyme activities in most cases. By comparing the kinase activities with different substrates, 32 kinases were found that preferentially phosphorylate one or two substrates, and 27 kinases were shown to readily phosphorylate poly(Tyr-Glu). This latter result suggests that there are many more potential yeast tyrosine kinases than known previously. Because almost the entire yeast protein kinase collection was screened, it was possible to correlate the large-scale functional data with structural data. Indeed, the enzymes that phosphorylated poly(Tyr-Glu) often share common amino acid residues that lie near the catalytic region.

The microwell protein chip is particularly suitable for biochemical assays for several reasons (Table 1). Firstly, proteins in microwells are well separated, so cross-contamination is minimal. Secondly, proteins in wells are kept in liquid that reduces sample drying. Thirdly, this technology is very sensitive and only requires a tiny amount of protein in each assay. Protein purified from a 3 ml culture is usually enough to perform 20 kinase assays. Small-scale assays are extremely valuable when working with precious materials (e.g. combinatorial chemical or peptide libraries). Fourthly, it is easy to change buffers in these microwells or to add individual components to different wells without cross-contamination. Because many biochemistry assays require multiple steps and sophisticated detection methods, this technique is expected to be widely adopted to different assays and drug screening. Fifthly, protein chips are inexpensive. Once the mold is created, many chips can be repeatedly cast at a cost of only 8 US cents per chip. Finally, this technique is easy to automate. Coupled with the existing ink-jet microarray technology, it is conceivable to analyze 10,000–100,000 biochemical reactions in parallel.

### Applications and future directions

Protein-array technology provides a powerful and versatile tool for the genome-scale analysis of gene function, such as enzyme activity, protein–protein and protein–nucleic-acid interaction and small-molecule drug interactions, directly on the protein level [30,31]. Currently, the rate-limiting step is the production of large numbers of proteins: the ability to automate protein production using a 96-well format and

Figure 2



Application of protein microwell chips in kinase assays. (a) The surface of the microwell chip is activated with a crosslinker GPTS (3-glycidypropyltrimethoxysilane) and cured at 130°C for an hour. Protein substrates are added to the wells and incubated in a humidity chamber for three hours on ice. Wells are blocked with 1% BSA and

washed with tris-buffered saline (TBS) buffer before kinase, <sup>33</sup>P-γ-ATP and buffer are added. After incubation for 30 min at 30°C, the chips are washed, and exposed to a Molecular Dynamics phosphorimager. (b) An enlarged image of the phosphorylation results using poly (Glu-Tyr) as a substrate.

proteins fused to high-affinity tags will greatly expedite protein-chip development. High-density chips containing large sets of proteins or even entire proteomes will allow the high-throughput analysis of biochemical activities, protein-protein interactions and post-translational modifications, such as phosphorylation, dephosphorylation, protein methylation, and ubiquitination.

Coupled with mass-spectrometric identification, protein chips might also have wide application in drug discovery and protein-protein interactions [32]. Proteins and small-molecule ligands can be bound to proteins immobilized on a protein chip and the bound molecules identified using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry. The format of microwells makes them particularly well suited for this purpose. By analyzing large numbers of samples, molecules and proteins that specifically bind to many different proteins can

be identified. Small molecules can then be used to probe protein function; protein-protein interactions can be used to deduce molecular networks and pathways.

One area that will probably require technical improvement is the analysis of membrane proteins. A large fraction of proteins are likely to be membrane-bound. For example, it has been suggested that as many as one third (28.2%) of all yeast proteins are membrane proteins or secreted proteins [33]. Many of these proteins are active only in their lipid state, and thus it may be necessary to purify or reconstitute them with associated lipids. However, these problems should be surmountable. Vogel and co-workers [34\*] have been able to immobilize biotinylated membranes that contain the G-protein-coupled receptor rhodopsin on a gold-coated glass surface, and establish a functional assay for that protein. This procedure and/or similar ones should make it possible to analyze membrane proteins in a chip format.

Future developments might also lie in the area of protein attachment. Although most groups are currently adhering proteins to the microarray surface in a random fashion, it is technically feasible to attach proteins through their affinity tag. For example, arrays coupled to glutathione can be used to attach GST-fusion proteins. Affinity attachment is likely to result in a higher proportion of active protein.

## Conclusions

Because proteins are one step closer to biochemical activities than are genes, these studies will lead directly to new biological discoveries and paradigms. Various technologies have been developed to produce protein chips, and the robotic ink-jet printing platform is preferred for transferring proteins to chips [35]. The ready availability of the full human-genome sequence will push the application of protein-chip technology to analysis of the human proteome.

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Research Paper

# Profiling the specific reactivity of the proteome with non-directed activity-based probes

Gregory C. Adam <sup>a</sup>, Benjamin F. Cravatt <sup>b,\*</sup>, Erik J. Sorensen <sup>a</sup>

<sup>a</sup>*The Skaggs Institute for Chemical Biology and Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, USA*

<sup>b</sup>*The Skaggs Institute for Chemical Biology and Department of Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, USA*

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## Abstract

**Background:** The field of proteomics aims to characterize dynamics in protein function on a global level. However, several classes of proteins, in particular low abundance proteins, remain difficult to characterize using standard proteomics technologies. Recently, chemical strategies have emerged that profile classes of proteins based on activity rather than quantity, thereby greatly facilitating the analysis of low abundance constituents of the proteome.

**Results:** In order to expand the classes of proteins susceptible to analysis by activity-based methods, we have synthesized a library of biotinylated sulfonate esters and applied its members to complex proteomes under conditions that distinguish patterns of specific protein reactivity. Individual sulfonates exhibited unique profiles of proteome reactivity that in extreme cases appeared nearly orthogonal to one another. A robustly labeled protein was

identified as a class I aldehyde dehydrogenase and shown to be irreversibly inhibited by members of the sulfonate library.

**Conclusions:** Through screening the proteome with a non-directed library of chemical probes, diverse patterns of protein reactivity were uncovered. These probes labeled protein targets based on properties other than abundance, circumventing one of the major challenges facing contemporary proteomics research. Considering further that the probes were found to inhibit a target enzyme's catalytic activity, the methods described herein should facilitate the identification of compounds possessing both selective proteome reactivities and novel bioactivities. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Proteomics; Sulfonate; Activity-based; Probe; Aldehyde dehydrogenase

## 1. Introduction

With the complete sequence of the human genome nearly in hand, biological research is entering a new era in which experimental focus will shift from identifying novel genes to determining the function of gene products. In efforts to accelerate the functional analysis of products expressed by the genome, researchers have developed several technologies that permit the study of biomolecules collectively, rather than individually. These global experimental strategies include: (1) genomics, or the analysis of a cell's complete transcript repertoire (transcriptome) [1,2], and (2) proteomics, or the analysis of a cell's complete

protein repertoire (proteome) [3,4]. In the field of genomics, advances in gene array technologies have permitted researchers to compare in a single experiment the levels of several thousand transcripts across two or more test samples [1,2,5]. Recent studies have demonstrated how gene chips can be used to discover and predict new subclasses of cancer based on their distinctive patterns of gene expression [6,7]. Still, by measuring dynamics in mRNA abundance, genomics approaches offer only an indirect assessment of protein quantity and function. Considering that mRNA levels are often poor predictors of protein abundance [8,9], the degree to which genomics data are reflective of changes in protein function remains unclear.

Recently, proteomics initiatives have emerged with the goal of characterizing dynamics in the abundances of proteins themselves [3,4,10]. Considerable experimental challenges face the analysis of the proteome, especially when compared to the technically more feasible profiling of the

\* Correspondence: Benjamin F. Cravatt and Erik J. Sorensen;  
E-mail: cravatt@scripps.edu  
E-mail: sorensen@scripps.edu

transcriptome. Unlike mRNA molecules, proteins (1) display tremendous chemical diversity (e.g. various co- and post-translational modifications, hydrophobic domains, etc.), (2) are not amplifiable, and (3) do not typically possess defined high affinity and high selectivity binding partners. Thus, perhaps not surprisingly, a general experimental method for total proteome analysis remains an elusive goal. To date, most proteomics studies have relied on two-dimensional gel electrophoresis (2DE) and mass spectrometry (MS) as their separation and detection technologies, respectively [11]. Advances in 2DE-MS methods have increased their reproducibility and throughput for proteome analysis [12]. However, a significant fraction of the proteome still remains difficult to analyze by 2DE, including very large and small proteins, very basic and acidic proteins, membrane proteins, and low abundance proteins [11-13]. In order to permit the general and systematic profiling of such 2DE-resistant proteins, proteomics researchers have proposed that alternative protein separation technologies must be developed [11,13]. Regardless, even if the requisite advances in separation strategies are achieved, it is critical to recognize that as long as proteomics focuses on measuring exclusively changes in protein abundance, it will provide, like genomics, only an indirect assessment of dynamics in protein function. Numerous post-translational events that regulated protein activity, especially those mediated by protein-protein and/or protein-small molecule interactions, will remain undetected by standard protein abundance-based techniques.

We have initiated a research program aimed at generating chemical probes that profile components of the proteome in an activity-dependent manner. In their broadest application, such activity-based probes (ABPs) would detect: (1) a significant, but manageable fraction of the proteome, (2) low abundance proteins and proteins with problematic biochemical properties (e.g. membrane proteins), and (3) dynamics in the activity of proteins independent of changes in their quantity. A prototype agent, a biotinylated fluorophosphonate (FP-biotin), was recently synthesized and shown to serve as an ABP for the serine hydrolase superfamily of enzymes [14]. Similarly, biotinylated epoxides and electrophilic ketones have been used to profile subclasses of the cysteine protease family [15-17]. In each of these examples, researchers exploited a wealth of previous knowledge on class-selective reactive groups and/or molecular scaffolds to create ABPs with predictable proteome reactivities (e.g. FPs for profiling serine hydrolases). However, for a number of protein families, selective active site-directed affinity reagents have not yet been identified, and whether such classes of proteins can be profiled in the manner illustrated above remains uncertain. Herein, we describe a non-directed approach for discovering new ABPs that bear both reactivities and selectivities compatible with whole proteome analysis. Additionally, we show that these chemical probes directly affect the activity of their target proteins, highlighting their potential utility in

function-based screens as well as proteomics investigations.

## 2. Results

### 2.1. Selection and synthesis of biotinylated sulfonate esters as candidate ABPs

In order to facilitate the discovery of new ABPs, we adopted a non-directed strategy for profiling the chemical reactivity of the proteome. A library of candidate ABPs was synthesized based on the general scaffold outlined in Fig. 1. The structure of an ABP was conceptually divided into four pieces: a binding group (BG), a reactive group (RG), a linker (L), and a tag (T). The library's reactive group was selected as a sulfonate ester, based on the following criteria. Sulfonates are: (1) moderately reactive electrophiles, (2) relatively unexplored as protein labeling reagents in biological systems, and (3) facile to modify with a variety of chemical structures. Additionally, we hoped that by choosing a carbon electrophile as the library's reactive group, its bias towards particular enzyme classes would be minimized. Indeed, other carbon electro-

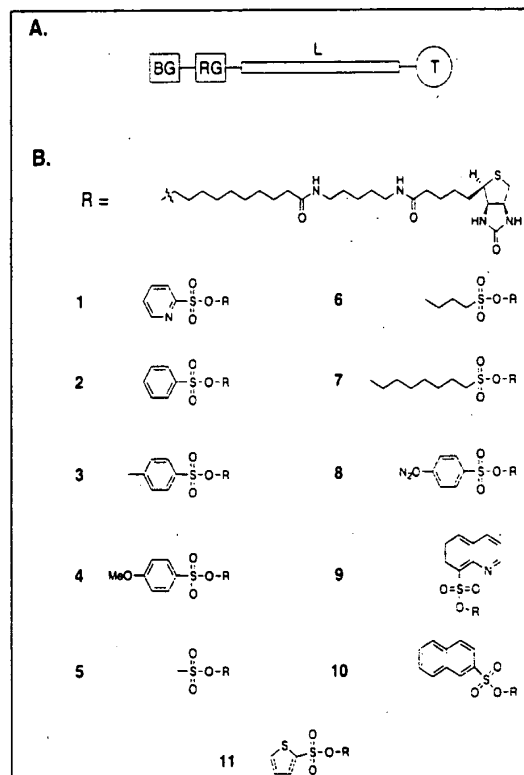


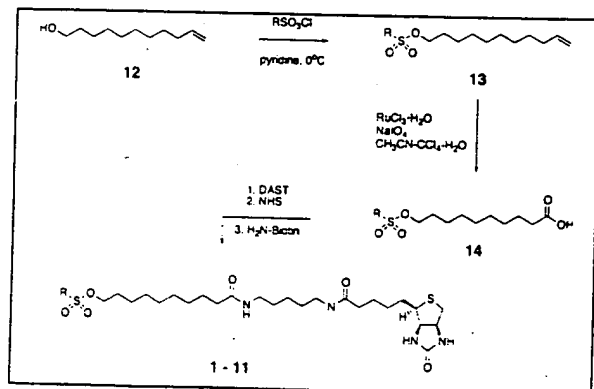
Fig. 1. A: General structure of an ABP, highlighting the probe's four main components: a binding group (BG), a reactive group (RG), a linker (L), and a tag (T). B: Structures of members of an ABP library, where the BG is varied, the RG is a sulfonate ester, the L is an extended alkyl chain, and the T is biotin.

philes like epoxides have been found to engage a large variety of enzymes through the alkylation of one of several different types of active site amino acids, including cysteine hydrolases (cysteines) [17], metallohydrolases (histidines) [18–20], glycosidases (glutamates/aspartates) [21], and proteosomal subunits (N-terminal amines) [22]. The library's binding group was combinatorially varied, while its linker moiety was kept constant and served as a bridge to the chemical tag (biotin). Through varying the binding group, we hoped to direct the sulfonate's specific proteome reactivity to different proteins and/or protein classes.

A series of biotinylated sulfonates (1–11; Fig. 1) were synthesized according to the three step sequence outlined in Scheme 1. The corresponding aryl or alkylsulfonyl chloride was added slowly to a solution of undecene-1-ol (12) dissolved in pyridine at 0°C to form the sulfonate (13). The procedure of Sharpless and colleagues was utilized to oxidatively cleave the terminal olefin, resulting in formation of the corresponding carboxylic acid (14) [23]. Treatment of 14 with diethylaminosulfur trifluoride followed by the addition of *N*-hydroxysuccinimide afforded the *N*-hydroxysuccinimidyl ester intermediate. The latter compound was reacted with commercially available 5-(biotinamido)pentylamine (NH<sub>2</sub>-biotin, Pierce) in methanol to form the desired biotinylated sulfonate ester.

## 2.2. Evaluating the specific proteome reactivity of biotinylated sulfonates

To determine whether members of the sulfonate library specifically targeted proteins in the proteome, a method was developed to rapidly distinguish a probe's specific and non-specific proteome reactivities. Each sulfonate (5 μM) was reacted with two versions of a rat testis proteome: a native proteome and a denatured proteome (generated by preheating the protein sample for 5 min at 80°C). After 30 min at 25°C, the sulfonate–proteome reactions were quenched by adding one reaction volume of standard sodium dodecyl sulfate (SDS)–PAGE loading buffer and analyzed by SDS–PAGE and avidin blotting.



Scheme 1. Synthetic route for the generation of biotinylated sulfonate esters.

A sulfonate's specific and non-specific proteome reactivity were defined as those protein targets that displayed heat-sensitive and heat-insensitive labeling, respectively. All sulfonates except the octylsulfonate 7 labeled at least one member of the testis proteome in a heat-sensitive manner (Fig. 2A,B). Interestingly, four general patterns of specific proteome reactivity were observed among the sulfonates. The *p*-toluenesulfonate 3, butylsulfonate 6, and naphthylsulfonate 10 each specifically labeled one 55 kDa protein (Fig. 2A,B; single arrowhead). The *p*-methoxybenzenesulfonate 4 and methylsulfonate 5 labeled four members of the proteome in a heat-sensitive manner (Fig. 2A; 55 kDa (single arrowhead), 42 kDa (double arrowhead), 40 kDa (triple arrowhead), and 32 kDa (single dot)), but also showed significant additional reactivity with the preheated proteome. The benzenesulfonate 2 reacted with three of the four proteins labeled by 4 and 5, failing to specifically label only the 40 kDa protein. The *p*-nitrobenzenesulfonate 8, quinolinesulfonate 9, and thiophenesulfonate 11 each reacted specifically with a 55 kDa protein, as well

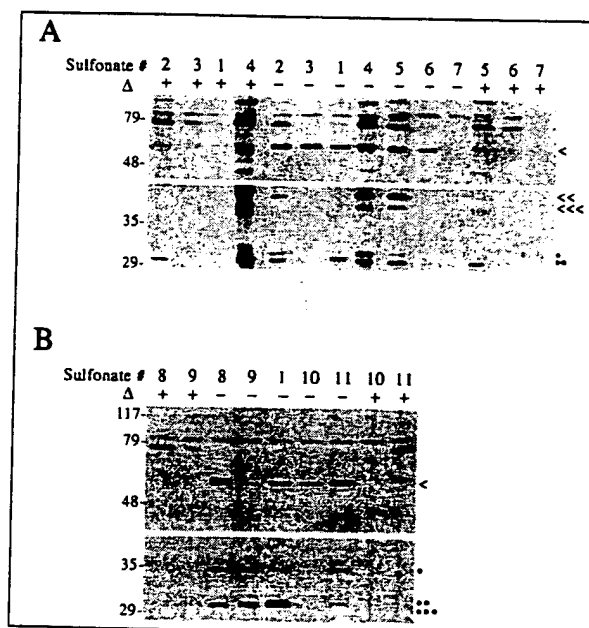


Fig. 2. Specific and non-specific proteome reactivities of sulfonates 1–7 (A) and 8–11 (B). For A and B, each sulfonate's reactivity with both a heated and unheated version of a rat testis proteome is shown (standard reaction conditions: 5 μM sulfonate, 0.5 μg/μl testis protein, 50 mM Tris–HCl, pH 8.0, 100 mM NaCl; 30 min reaction, 25°C). Sulfonate-labeled proteins were detected by SDS–PAGE (7.5 μg protein/lane) and avidin blotting. Highlighted with arrowheads and dots are proteins that reacted with sulfonates in a heat-sensitive manner (see text). The proteins labeled in the lanes containing preheated proteomes (Δ) were all considered 'non-specific' sulfonate reactivities, except an 80 kDa protein which represented an endogenous avidin-reactive protein (i.e. also observed in proteomes not treated with sulfonates; see Fig. 4B). Different film exposure times are presented for the high (45–100 kDa, 1× time exposure) and low (27–45 kDa, 4× time exposure) molecular mass proteins to permit the signals of labeled proteins to be shown prior to film saturation.

as two additional proteins poorly labeled by the other reagents (Fig. 2B; 36 kDa (single dot) and 30 kDa (triple dot)). Finally, the pyridylsulfonate 1 labeled a 55 kDa protein and a 31 kDa protein (Fig. 2A,B, single arrowhead and double dot, respectively), the latter protein appearing uniquely reactive with 1 among the sulfonate probes with the exception of 2, 4, and 5 showed low or negligible reactivity with the preheated proteome.

Although several sulfonate probes showed overlapping patterns of specific proteome reactivity, their relative reactivities with individual proteins differed considerably. For example, a 36 kDa protein reacted more strongly with quinolinesulfonate 9 than with pyridylsulfonate 1, *p*-nitrobenzenesulfonate 8 or thiophenesulfonate 11, while a 55 kDa protein displayed the opposite probe selectivity (Fig. 2B). To further examine the different proteome reactivities exhibited by individual sulfonates, the labeling pattern of pyridylsulfonate 1 was compared to that of methylsulfonate 5 and quinolinesulfonate 9 in side-by-side analyses (Fig. 3A,B, respectively). Sulfonates 1 and 5 exhibited quite distinctive patterns of specific proteome reactivity,

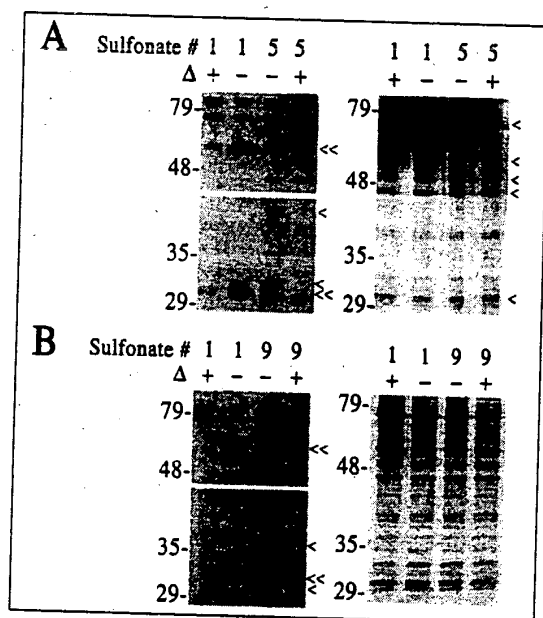


Fig. 3. Side-by-side comparisons of the proteome reactivities of sulfonates 1, 5, and 9. A: Left panel, proteome reactivities of sulfonates 1 and 5. Heat-sensitive protein reactivities selective for 1 and 5 are highlighted (double and single arrowheads, respectively). Right panel, Coomassie blue-stained protein gel of samples treated with 1 and 5. Arrowheads highlight abundant proteins correlating in molecular mass with proteins labeled by 5 in the preheated proteome. B: Left panel, proteome reactivities of sulfonates 1 and 9. Heat-sensitive protein reactivities selective for 1 and 9 are highlighted (double and single arrowheads, respectively). Right panel, Coomassie blue-stained protein gel of samples treated with 1 and 9. For A and B, different film exposure times are presented for the high (45–100 kDa, 1× time exposure) and low (27–45 kDa, 4× time exposure) molecular mass proteins to permit the signals of labeled proteins to be shown prior to film saturation.

with 1 most strongly labeling 55 and 31 kDa proteins (Fig. 3A, left panel, double arrowheads) and 5 most strongly labeling 42 and 32 kDa proteins (Fig. 3A, left panel; single arrowheads). A comparison of the proteome reactivities of 1 and 9 identified two proteins that showed preferred reactivity with 1 (Fig. 3B, left panel, 55 kDa and 31 kDa; double arrowheads) and two proteins that showed enhanced reactivity with 9 (Fig. 3B, left panel, 36 kDa and 30 kDa; single arrowheads).

The greater non-specific reactivity of 5 was also evident in these side-by-side comparisons, as this agent labeled several proteins in a heat-insensitive manner that were unreactive towards 1 and 9. Notably, a Coomassie blue-stained protein gel revealed that the proteins labeled by 5 in the preheated proteome represented very abundant proteins (Fig. 2C, right panel, single arrowheads), consistent with the notion that heat-insensitive labeling reflects a non-specific form of sulfonate reactivity. In contrast, the sulfonate library's heat-sensitive proteome reactivity showed no such bias towards abundant proteins. Finally, it is interesting to note that little correlation was observed between the magnitudes of a sulfonate's specific and non-specific forms of proteome reactivity, as several probes displaying low reactivity with the preheated proteome exhibited specific reactivities that equaled or exceeded in intensity those exhibited by probes with high non-specific reactivities.

### 2.3. Parameters that influence a sulfonate's specific proteome reactivity

The following features of the sulfonate-proteome reaction were varied in order to test their influence on the observed specific and non-specific protein labeling patterns: time, sulfonate concentration, pH, and the presence/absence of scavenging nucleophiles. For these studies, the reactivity of pyridylsulfonate 1 with the testis proteome was examined. The two testis proteins specifically targeted by 1 were labeled at similar rates, with their signal intensities increasing from 5 to 40 min and then plateauing from 40 to 120 min (Fig. 4A). The absence of additional reactivity from 40 to 120 min could signify that the proteins had labeled to completion by 40 min, or alternatively, that the concentration of 1 in the reaction was significantly depleted by these later time points (this latter explanation seems less likely when taking into account that pyridylsulfonates were quite stable to the assay conditions employed; see below).

Sulfonate 1's specific and non-specific proteome reactivities were evaluated over a range of probe concentrations (1–50  $\mu$ M). From 1 to 10  $\mu$ M, sulfonate 1 showed specific reactivity with the 31 and 55 kDa proteins that increased in intensity with increasing concentrations of reagent (Fig. 4B). Over this concentration range, sulfonate 1 displayed very low levels of heat-insensitive reactivity with the proteome. From 10 to 50  $\mu$ M of 1, the signal intensity of the

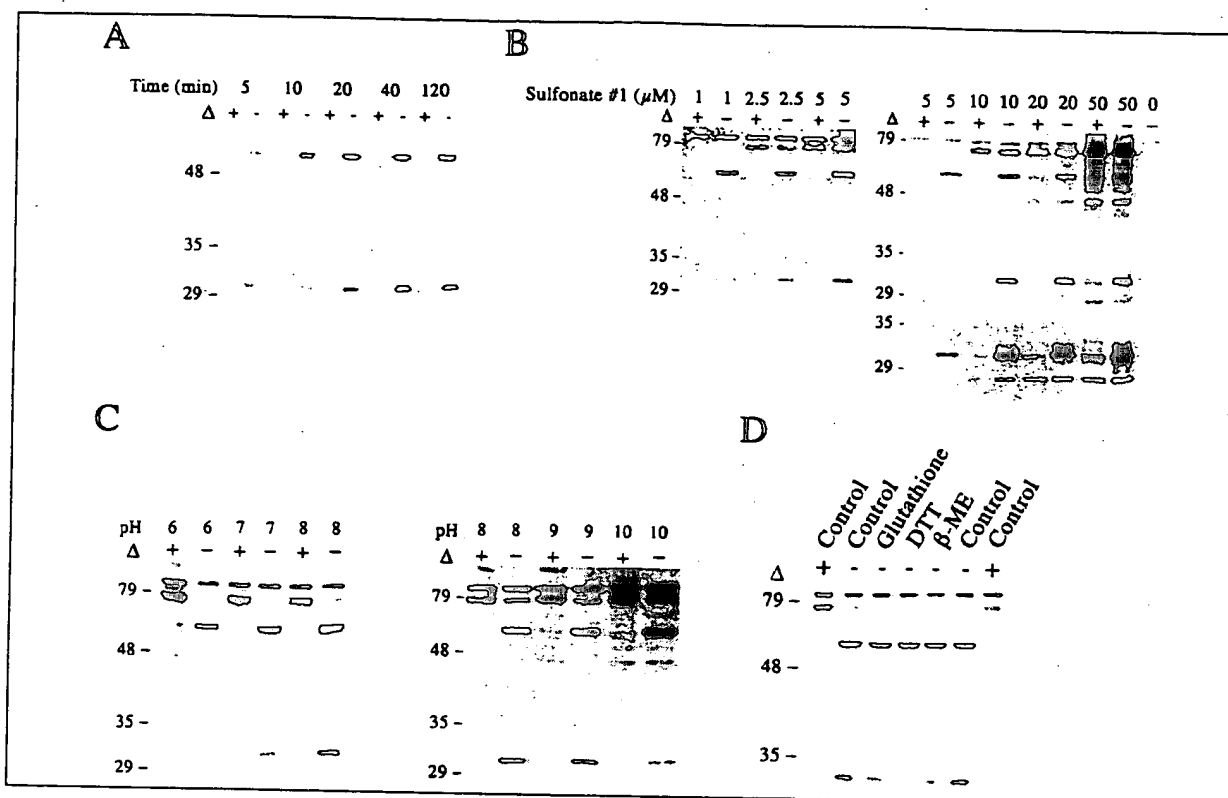


Fig. 4. Parameters that affect the proteome reactivity of pyridylsulfonate 1. A: Time-dependence of 1-proteome reaction (5  $\mu$ M of 1, 0.5  $\mu$ g/ $\mu$ l protein, 50 mM Tris-HCl, pH 8.0). B: Concentration-dependence of 1-proteome reaction. Left panel, 1, 2.5, and 5  $\mu$ M concentrations of 1 were reacted with the testis proteome (0.5  $\mu$ g/ $\mu$ l protein, 50 mM Tris-HCl, pH 8.0, 30 min reaction). Right panel, 5, 10, 20, and 50  $\mu$ M concentrations of 1 were reacted with the testis proteome (0.5  $\mu$ g/ $\mu$ l protein, 50 mM Tris-HCl, pH 8.0, 30 min reaction). Short (upper right panel) and long (lower right panel) film exposures of these reactions are shown. Note the presence of an endogenous 80 kDa avidin-reactive protein in the lane containing an untreated testis proteome. C: pH-dependence of 1-proteome reaction (5  $\mu$ M of 1, 0.5  $\mu$ g/ $\mu$ l protein, 30 min reaction). Left panel, reactions conducted from pH 6.0 to 8.0. Right panel, reactions conducted from pH 8.0 to 10.0. D: Thiol-dependence of 1-proteome reaction. Control reactions were conducted under standard conditions. Each thiol (2 mM) was added to the proteome prior to the addition of 1.

31 kDa protein continued to increase, while the intensity of the 55 kDa protein remained relatively constant. Over this concentration range, sulfonate 1's non-specific labeling increased dramatically, especially in the higher molecular mass range where most of the abundant testis proteins reside. Importantly, however, no new specifically labeled protein targets were identified over this concentration range. Thus, a concentration range of 5–10  $\mu$ M appeared optimal for maximizing sulfonate 1's specific versus non-specific proteome reactivity.

The non-specific and specific proteome reactivities of sulfonate 1 showed different pH-dependencies, with the former appearing as an inverted bell-shape curve (higher background labeling at pH 6 and 9 than at pH 7 and 8) and the latter increasing in intensity from pH 6 to 8 and plateauing from pH 8 to 10 (Fig. 4C,D). Thus, reactions conducted at pH 7 and 8 produced the highest level of specific reactivity, while at the same time resulting in the lowest degree of non-specific reactivity.

Sulfonate 1's intrinsic reactivity with nucleophiles was examined by conducting proteome reactions in the pres-

ence of millimolar concentrations of free thiols (glutathione,  $\beta$ -mercaptoethanol, or dithiothreitol (DTT)). If this sulfonate displayed a high reactivity with generic nucleophiles, then the probe's effective concentration in thiol-treated proteome reactions should be greatly reduced, resulting in a significant decrease in the signal intensity of specifically labeled proteins. However, none of the tested thiols affected the labeling intensity of the 55 kDa protein, indicating that sulfonate 1's intrinsic reactivity with nucleophiles is low (Fig. 4D; see below for more evidence in support of this notion). In contrast, a moderate decrease in the labeling intensity of the 31 kDa protein was detected in the presence of free thiols, possibly indicating that this protein's structure/activity is sensitive to these reagents (e.g. if this protein's tertiary structure is stabilized by disulfide bonds).

#### 2.4. Molecular identification of a protein labeled by biotinylated sulfonates

The screening method described above was enacted to

rapidly identify protein targets specifically labeled by members of the sulfonate library. By defining these 'specific protein targets' as ones that displayed heat-sensitive reactivity with sulfonates, we hoped to restrict our focus to proteins whose activities would be affected by sulfonate labeling. The assumption inherent to this strategy was that heat-sensitive labeling was reflective of an event taking place within a structured portion of a protein suitable for small molecule binding. Such structures were anticipated to often represent either ligand binding pockets of receptors or active sites of enzymes. As such, if a sulfonate reacted specifically with one of these sites on a receptor or an enzyme, its reaction might in turn be expected to affect the activity of this protein. In order to test this premise, we pursued the molecular identification of the 55 kDa protein specifically labeled by several members of the sulfonate library.

A tissue blot with sulfonate 1 revealed that the labeled 55 kDa protein was most abundant in soluble fractions of rat liver (data not shown), and therefore the protein was purified from this source. The 55 kDa protein was partially purified by Q-Sepharose anion exchange chromatography. Aliquots of the flow-through and elution fractions of this column were labeled with 1, and the 55 kDa protein was identified in the flow-through fractions. These fractions were combined, labeled with 5  $\mu\text{M}$  1 for 30 min, and the protein separated from excess sulfonate by size exclusion chromatography. The protein sample was then treated with avidin agarose beads to isolate the 1-labeled 55 kDa protein. Elution of the avidin-bound proteins was achieved by adding one volume of standard SDS-PAGE loading buffer and heating (90°C, 5 min). This avidin-based affinity purification procedure provided a highly concentrated sample of the 55 kDa protein that was separated by SDS-PAGE and either blotted with avidin (Fig. 5A) or stained with Coomassie blue. The 55 kDa protein was excised from the stained gel, treated with trypsin, and the resulting peptide mixture analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS. MS-Fit and ProFound searches of protein databases identified the protein as cytosolic 2 class I aldehyde dehydrogenase (cALDH-I; nine tryptic peptides ranging from 1189 to 2055 Da matched this enzyme, 50% total sequence coverage; Fig. 5A), a member of a superfamily of NAD<sup>+</sup>-dependent enzymes responsible for the oxidation of endogenous and exogenous aldehydes to carboxylic acids [24,25].

## 2.5. Recombinant expression of cALDH-I

In order to confirm the specific reactivity of cALDH-I with sulfonate 1, this protein was recombinantly expressed in both eukaryotic and prokaryotic systems. The cALDH-I cDNA was subcloned into the pcDNA3 mammalian expression vector and then transfected into COS-7 and MCF-7 cells. cALDH-I-transfected COS-7 and MCF-7 cells both expressed a 55 kDa protein that labeled strongly

with sulfonate 1 (Fig. 5B). In contrast, this sulfonate-reactive protein was not detected in mock-transfected versions of each cell type. cALDH-I was also recombinantly expressed in *Escherichia coli* using the pTrcHis system. Lysates from cALDH-I-transformed *E. coli* were treated with sulfonate 1 and found to express a single reactive protein of the predicted size for the cALDH-I enzyme bearing an N-terminal histidine tag (60 kDa; Fig. 5C). The His-tagged cALDH-I was purified from *E. coli* lysates by sequential metal affinity and gel filtration chromatography. This prokaryotic expression system routinely provided 15 mg/l culture volume of purified cALDH-I enzyme.

Members of the sulfonate library displayed a relatively broad range of apparent reactivities with cALDH-I in the soluble testis proteome, with some probes labeling the 55 kDa enzyme quite strongly and others displaying little or no reactivity towards this protein (see Fig. 2). In order to determine whether the sulfonate labeling events observed for the 55 kDa protein in the testis proteome reflected reactivity primarily with cALDH-I, several sulfonate probes were incubated with cALDH-I-transfected COS-7 extracts. Interestingly, the relative reactivities of sulfonates with the COS-expressed cALDH-I enzyme matched closely their relative labeling efficiencies with the 55 kDa testis protein (Fig. 5D), indicating that the sulfonate labeling pattern observed at this molecular mass in the testis proteome was predominantly due to cALDH-I.

## 2.6. Pyridylsulfonates are time-dependent inhibitors of cALDH-I catalytic activity

cALDH-I-catalyzed oxidation of propionaldehyde to propionic acid was measured by observing the reduction of NAD<sup>+</sup> to NADH at 340 nm. The observed Michaelis constant for propionaldehyde ( $K_m = 4.2 \mu\text{M}$ ) displayed by the His-tagged recombinant cALDH-I was comparable to the reported literature value for this enzyme ( $K_m = 6.5 \mu\text{M}$ ) [26]. To examine the effect of sulfonates on cALDH-I's catalytic activity, the enzyme was treated with varying concentrations of 2-pyridylsulfonyl octanoate (15), a variant of 1 lacking the probe's biotin tag. Sulfonate 15 inhibited cALDH-I's catalytic activity in a time-dependent manner that increased in rate from 2.5 to 15  $\mu\text{M}$  inhibitor (Fig. 6A). Concentrations of 15 greater than 15  $\mu\text{M}$  inactivated cALDH-I at a rate that was too fast to measure under the assay conditions employed. The average  $K_{obs}/[I]$  value calculated from reactions conducted at 2.5, 5.0, 7.5, 10, and 15  $\mu\text{M}$  15 was  $(9.7 \pm 1.8) \times 10^3 \text{ M}^{-1} \text{ min}^{-1}$ . Although the inactivation of cALDH-I at each concentration of 15 could be fit to pseudo-first order kinetics, extrapolation of these reactions back to time zero did not predict 100% enzyme activity. Considering that cALDH-I is a homotetrameric protein [27], one possible explanation for these data was that individual cALDH-I subunits exhibited different rates of reactivity with 15. In general sup-



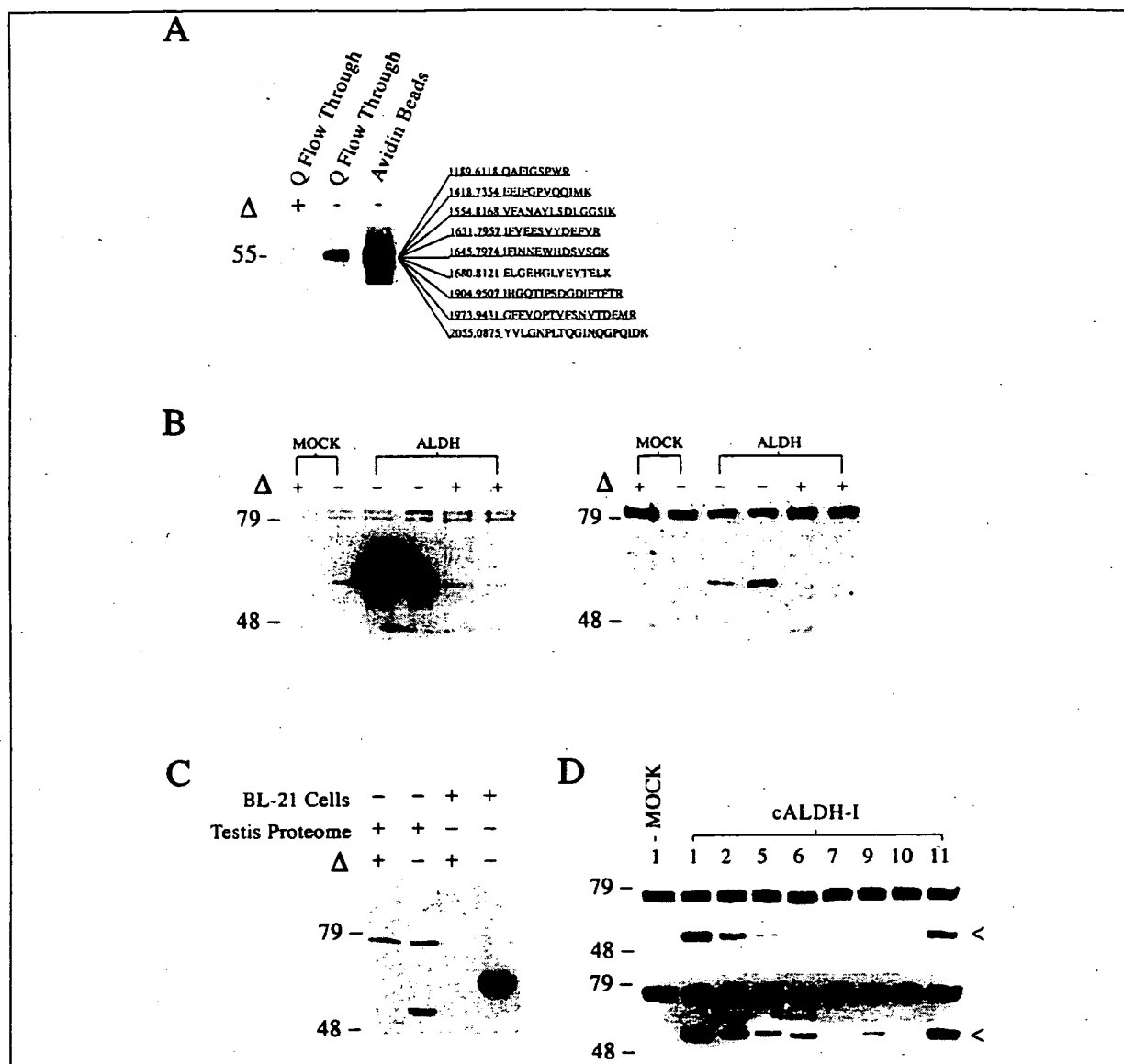


Fig. 5. Identification of a 55 kDa specifically labeled sulfonate target as cALDH-I. A: Avidin-based affinity isolation of the 55 kDa 1-labeled protein. Shown is an avidin blot of samples containing the partially purified 55 kDa protein (Q Flow Through) and the affinity-isolated 55 kDa protein (Avidin Beads). Also shown are the tryptic peptides from this protein that identified it as cALDH-I. B: Sulfonate 1 labels recombinant cALDH-I in eukaryotic expression systems. Protein samples from COS-7 (left panel) and MCF-7 (right panel) cells transfected with the cALDH-I cDNA or empty vector (mock) were reacted with 1 and resolved by SDS-PAGE and avidin blotting. A strongly labeled 55 kDa protein was identified only in the cALDH-I-transfected cells. C: Sulfonate 1 labels recombinant cALDH-I in prokaryotic expression systems. A protein sample of *E. coli* BL-21 cells transformed with a His-tagged version of cALDH-I was reacted with 1 and resolved by SDS-PAGE and avidin blotting. A strongly labeled 60 kDa protein was identified, corresponding to the predicted molecular mass of cALDH-I with an appended N-terminal histidine tag. D: Reactivity of recombinantly expressed cALDH-I with members of the sulfonate library. Sulfonate probes (numbers shown above lanes) were reacted with cALDH-I-transfected COS-7 extracts and the reactions comparatively analyzed by SDS-PAGE and avidin blotting. A mock-transfected COS extract treated with sulfonate 1 was also run as a control. Note that the relative reactivity of sulfonates with the COS-expressed cALDH-I enzyme (arrowhead) matched closely their relative labeling efficiencies with the 55 kDa protein of the testis proteome (Fig. 2). Top and bottom panels represent 1 and 5 min film exposures, respectively.

port of this notion, the kinetics of reactions conducted at lower concentrations of 15 appeared biphasic in nature, with time points preceding 50% enzyme inhibition predicting a slightly faster rate of cALDH-I inactivation than time points at which greater than 50% cALDH-I activity

was inhibited. Finally, incubating 15 in the reaction buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM DTT) for 60 min prior to the addition of cALDH-I did not affect the inhibitor's potency, indicating that this sulfonate was

stable to the assay conditions employed (including the presence of excess free thiols).

To probe the nature of **15**'s interaction with cALDH-I, competition studies were performed with both propionaldehyde and NAD<sup>+</sup>. Recombinant cALDH-I was treated with 10  $\mu$ M **15** for 10 min either in the presence or absence of 25  $\mu$ M propionaldehyde or 50  $\mu$ M NAD<sup>+</sup> and the percentage of enzyme activity remaining was determined (Table 1). Propionaldehyde had no detectable effect on **15**'s inactivation kinetics. In contrast, 50  $\mu$ M NAD<sup>+</sup> significantly reduced **15**'s inhibition of cALDH-I, and higher concentrations of NAD<sup>+</sup> completely protected the enzyme from inactivation (data not shown).

### 2.7. Features of pyridylsulfonates responsible for cALDH-I inactivation

Analogues of **15** were synthesized in which the agent's octyl and pyridyl substituents were replaced with ethyl (**16**) and methyl (**17**) groups, respectively. cALDH-I was incubated for 60 min with 50  $\mu$ M of either **15**, **16**, or **17**, and the percentage of cALDH-I activity remaining was determined. While **15** completely inactivated cALDH-I under these conditions, **16** and **17** produced weak and no inhibition, respectively. A  $K_{\text{obs}}/[I]$  value of 0.25 M<sup>-1</sup> min<sup>-1</sup> was calculated for **16**, representing a second order inhibition rate constant 40 000 times lower than that determined for **15**. **15**–**17** were also tested for their ability to block sulfonate **1**'s reactivity with cALDH-I in the soluble testis proteome. The testis proteome was preincubated for 30 min with each non-biotinylated sulfonate at concentrations of 5 or 50  $\mu$ M. The proteome samples were then treated with 5  $\mu$ M **1** and the reaction mixtures incubated for 30 min prior to analysis by SDS-PAGE and avidin blotting. Consistent with the inhibition kinetics described above, only **15** blocked the labeling of cALDH-I by **1** in the testis proteome (Fig. 6B). Collectively, these data highlight that the interaction of sulfonate **1** with cALDH-I depends on the chemical nature of both the linker and binding groups of the inhibitor.

### 2.8. Multiplexing ABPs increases proteome coverage

Although individually, biotinylated sulfonates only profiled a modest fraction of the proteome, their application together, or in combination with other ABPs ('multiplexing') may significantly augment the number of proteins

visualized in a single assay. To test this notion, the rat testis proteome was treated with either 2.5  $\mu$ M sulfonate **1**, 5  $\mu$ M FP-biotin, or a mixture of 2.5  $\mu$ M **1** and 5  $\mu$ M FP-biotin [14], and the resulting heat-sensitive labeling profiles were visualized by SDS-PAGE and avidin blotting. As can be seen in Fig. 7, applying a mixture of sulfonate **1** and FP-biotin to the testis proteome effectively detected in a single sample the proteins labeled by both probes individually. Notably, a preheated sample treated with the same probe mixture displayed a very low level of labeling that was comparable to the non-specific reactivity observed when each probe was tested alone. These data suggest that by multiplexing ABPs, one can significantly increase the coverage of specific protein reactivities detectable in a single proteome assay.

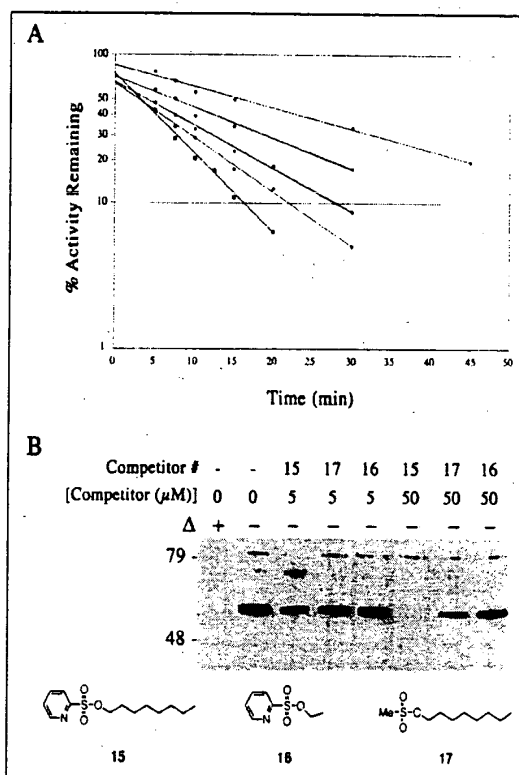


Fig. 6. Sulfonates are time-dependent inhibitors of cALDH-I. A: Time-dependent inactivation of cALDH-I as a function of sulfonate **15** concentration. Recombinant, purified cALDH-I was incubated with different concentrations of **15** and at the time points shown, aliquots of the reaction were removed and assayed for enzyme activity using 1 mM propionaldehyde and 0.5 mM NAD<sup>+</sup> as substrate and cofactor, respectively. Concentrations of **15** were: solid diamonds, 2.5  $\mu$ M; hollow diamonds, 5  $\mu$ M; solid circles, 7.5  $\mu$ M; hollow circles, 10  $\mu$ M; solid squares, 15  $\mu$ M. B: Competition reactions between sulfonate **1** and structural analogs **15**–**17**. Sulfonate **15** effectively blocked the labeling of cALDH-I by **1** in the testis proteome. Analogs of **15** in which this sulfonate's pyridyl and octyl groups were replaced with methyl and ethyl groups, respectively (**16** and **17**, respectively), did not block the labeling of cALDH-I by **1**.

Table 1  
Competition studies for **15** with propionaldehyde and NAD<sup>+</sup> in cALDH

Substrate	Sulfonate <b>15</b> ( $\mu$ M)	Activity remaining (%)
–	–	100
–	5	15
50 $\mu$ M NAD <sup>+</sup>	5	61
25 $\mu$ M Propionaldehyde	5	14

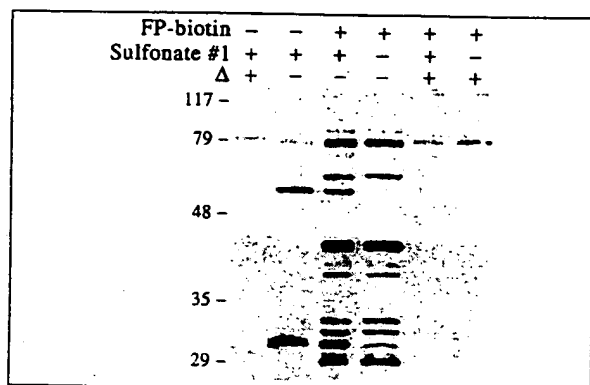


Fig. 7. Multiplexing ABPs increases the number of protein activities detected in a single proteome. Shown is a comparison of the heat-sensitive labeling patterns of a testis proteome treated with pyridylsulfonate 1 (2.5  $\mu$ M), FP-biotin (4  $\mu$ M), or a mixture of pyridylsulfonate 1 (2.5  $\mu$ M) and FP-biotin (4  $\mu$ M). The mixture-treated proteome exhibited a labeling profile similar to that predicted from merging the profiles of the proteome treated with each ABP alone.

### 3. Discussion

Genomics and proteomics aim to characterize on a global level changes in the expression of genes and proteins, respectively. Both methods generate data that are anticipated to reflect dynamics in molecular and cellular function. Considering that proteins are the main components of the cell responsible for executing its various activities, proteomics data could be viewed as bringing one conceptually closer to the goal of understanding complex cellular function. However, proteomics initiatives face serious methodological challenges that have limited their impact, especially when compared to the technically more feasible field of genomics. To date, the field of proteomics has relied primarily on 2DE and MS as its separation and detection methods, respectively [11–13]. Although a significant fraction of the proteome can be analyzed with 2DE-MS methods, numerous classes of proteins remain undetected, including very large/small, highly basic/acidic, membrane, and low abundance proteins [11–13]. Moreover, by measuring changes in protein abundance, standard proteomics experiments provide only an indirect assessment of dynamics in protein activity.

We have initiated a research program aimed at generating chemical probes that profile components of the proteome in an activity-dependent manner. For example, a biotinylated fluorophosphonate (FP-biotin) was recently described that acts as an ABP for the serine hydrolase superfamily of enzymes [14]. Similarly, other researchers have generated biotinylated epoxides and electrophilic ketones as profiling agents for subclasses of cysteine proteases [15–17]. Each of these ABPs measures changes in enzyme activity directly in complex proteomes, thereby offering a global visualization of dynamics in protein function independent of changes in protein abundance. Addi-

tionally, an ABP's biotin tag serves not only as a sensitive detection device for classes of low abundance and biochemically problematic proteins (e.g. membrane proteins; our unpublished data), but also as a means for rapidly isolating and identifying labeled proteins using avidin-based solid supports.

In the design of ABPs that targeted serine and cysteine hydrolases, researchers exploited a set of thoroughly characterized active site-directed reactive groups and/or chemical structures. However, for many enzyme and protein families, well-defined affinity labeling reagents have not yet been identified. In order to expedite the discovery of new ABPs that show protein selectivities and reactivities compatible with complex proteome analyses, we have synthesized a library of biotinylated sulfonate esters and applied these agents to complex proteomes using an assay that distinguished their specific and non-specific protein reactivities. This assay involved comparing a sulfonate's reactivity with native and heat-denatured versions of the proteome. Proteins that showed heat-sensitive sulfonate reactivity were considered 'specific protein targets', while those that were labeled in both native and heat-denatured proteomes were defined as 'non-specific protein targets'. We hypothesized that proteins reacting with members of the sulfonate library in a heat-sensitive manner would likely possess structured sites for small molecule interaction, and that these sites would often determine the biological activity of the protein (e.g. ligand binding pockets of receptors or active sites of enzymes). In such cases, sulfonate labeling was hoped to affect the function of the protein target.

Members of the sulfonate library displayed strikingly different specific and non-specific proteome reactivities. At low micromolar concentrations, most sulfonates exhibited heat-sensitive reactivity with a discrete number of proteins, while at the same time showing very low or negligible reactivity with the heat-denatured proteome. Individual sulfonates displayed unique patterns of specific reactivity, and even subtle variations in the sulfonate's binding group resulted in significant changes in proteome labeling. For example, the pyridyl (1), benzene (2), and *p*-tosyl (3) sulfonates, despite their structural similarity, all displayed distinct specific proteome reactivities, with 1 labeling 31 and 55 kDa proteins, 2 labeling 32, 42, and 55 kDa proteins, and 3 labeling exclusively a 55 kDa protein. A few sulfonates, like the *p*-methoxybenzenesulfonate 4, exhibited a high degree of non-specific reactivity, labeling numerous proteins in both heat-denatured and native proteomes. All of these non-specific targets represented proteins of high abundance (as judged by Coomassie blue staining), suggesting that a sulfonate's heat-insensitive labeling was more reflective of its intrinsic reactivity than a selective association with particular constituents of the proteome. In contrast, the sulfonate library's heat-sensitive labeling showed no such bias towards abundant proteins, indicating that these reactivities resulted from specific

small molecule-protein interactions. We speculate that an expanded library of ABPs in which not only the binding group, but also the linker and reactive group are varied will uncover additional probes that display distinct patterns of specific proteome reactivity.

Perhaps not surprisingly, the ratio of a sulfonate's specific versus non-specific reactivity depended strongly on the concentration of the agent applied to the proteome. For example, sulfonate **1** showed negligible heat-insensitive labeling at 1-5  $\mu\text{M}$  concentrations, but as its concentration approached 50  $\mu\text{M}$ , the probe began to label numerous proteins in both the native and heat-denatured proteomes. Interestingly, no additional heat-sensitive reactivities were detected when the concentration of **1** was raised from 5 to 50  $\mu\text{M}$ . Collectively, these data highlight the importance of determining for a given electrophilic probe the concentration range over which the agent's ratio of specific versus non-specific reactivity is maximized.

To examine the sulfonate library's specific proteome reactivities in more detail, a heat-sensitive 55 kDa protein target was isolated by avidin-based affinity chromatography and identified as cALDH-I. ALDHs constitute a superfamily of enzymes that utilize the cofactor  $\text{NAD}^+$  ( $\text{NADP}^+$ ) to oxidize exogenous and endogenous aldehydes to carboxylic acids through formation of a thioester intermediate with an active site cysteine nucleophile [28-30]. The ALDH family is divided into three subclasses: the homotetrameric cytosolic class I and the mitochondrial class II isoforms and the homodimeric cytosolic class III isoforms [25,27]. Genome sequencing projects have identified numerous ALDHs in *Drosophila melanogaster* (28 ALDHs), *Caenorhabditis elegans* (13 ALDHs), and *Saccharomyces cerevisiae* (13 ALDHs) [31]. In humans, at least 16 ALDH genes have already been characterized [32]. Mammalian ALDHs are noted for their roles in retinoic acid biosynthesis [33], the detoxification of nitrogen-mustard based chemotherapeutic agents [34-36], and the catabolism of ethanol to acetic acid [37]. cALDH-I, a quantitatively minor, though widely distributed cytosolic protein, appears to function in the retinoid signaling pathway by catalyzing the irreversible oxidation of retinal to retinoic acid [38,39].

The heat-sensitive reactivity of cALDH-I with sulfonate **1** was confirmed by expressing this enzyme in both eukaryotic and prokaryotic systems. Additionally, sulfonate **15** (a non-biotinylated version of **1**) was found to act as a time-dependent irreversible inhibitor of cALDH-I, exhibiting a  $k_{\text{obs}}/[\text{I}]$  value of  $9.7 \times 10^3 \text{ M}^{-1} \text{ min}^{-1}$  (similar inhibitory properties were observed for sulfonate **1**; data not shown). Excess  $\text{NAD}^+$  protected cALDH-I from inhibition by **15** (as well as from labeling by **1** in complex proteomes; data not shown), consistent with the notion that this sulfonate labeled cALDH-I at or near its active site. Analogs of **15** in which the agent's pyridyl and octyl groups were replaced with methyl and ethyl groups, respectively, were inactive as cALDH-I inhibitors, indicating that both the

binding group and linker components of **15** contributed significant binding interactions. Nonetheless, saturation kinetics could not be achieved with **15** even at concentrations as high as 20  $\mu\text{M}$ , indicating that its binding affinity for cALDH-I is relatively weak. These data, in conjunction with the recognition that sulfonate **1** reacted with only two proteins in the testis proteome, suggest that an ABP displaying a combination of tempered reactivity and modest binding affinity can engage in protein labeling events of high selectivity.

The relatively potent and selective inhibition of cALDH-I by **15** raises several intriguing issues. First, what is the mechanism by which **15** reacts with cALDH-I? Although it is attractive to speculate that the enzyme's cysteine nucleophile represents the site of sulfonate labeling, the cALDH-I-**15** adduct has not yet been isolated and characterized. If cALDH-I's nucleophile does prove to be the site of reactivity, then sulfonates appended with the appropriate binding group(s) could act as active site-directed profiling agents for the ALDH family as a whole. Indeed, considering that many ALDHs are 55 kDa in size, at present we cannot exclude the possibility that our sulfonate library is reacting with more than one ALDH in the proteome. Finally, sulfonates like **15** could serve as lead compounds for the generation of highly selective and potent inhibitors of ALDHs, an enzyme family of significant therapeutic importance. On this note, disulfiram, a relatively non-selective ALDH inhibitor, has been used clinically for many years in alcohol aversion therapy [40]. However, disulfiram's therapeutic utility is limited by its high reactivity in vivo, resulting in the non-specific carbamylation of several serum and cellular proteins [41,42] and glutathione [43]. Thus, more selective and potent ALDH inhibitors may find application in the treatment of alcoholism, as well as various forms of cancer [34-36].

#### 4. Significance

The chemical and biochemical approaches described herein represent an alternative strategy for analyzing the proteome that is both complementary and in some aspects superior to the standard 2DE-MS methods currently in practice. By treating complex proteomes with a library of chemical probes bearing a moderately reactive sulfonate ester, diverse patterns of specific protein reactivity were identified. Probes were characterized in terms of their heat-sensitive protein labeling patterns, permitting the discovery of reagents that profiled proteins based on properties other than abundance. Although such ABPs when applied individually to the proteome detected only a limited set of proteins, their utility in combination should greatly enhance the characterization of a large number of low abundance proteins. Finally, the discovery that sulfonate probes not only labeled cALDH-I in complex proteomes, but also inhibited this enzyme's catalytic activ-

ity suggests that, at least in this one example, a screen for heat-sensitive labeling events accurately identified a small molecule–protein reaction that impacted the protein's biological function. If this correlation proves generalizable, non-directed approaches for profiling the specific reactivity of the proteome may generate chemical reagents applicable for both proteomics investigations and cell-based functional screens. In this latter case, one could envision screening a library of ABPs for compounds that possess a particular cellular bioactivity, and then comparing the proteome labeling patterns of biologically active and inactive library members to identify the affected protein target(s).

## 5. Materials and methods

### 5.1. Synthesis alkyl and aryl sulfonate esters

All reactions were carried out under an atmosphere of argon unless specified. Methylene chloride ( $\text{CH}_2\text{Cl}_2$ ) was dried by passing through activated alumina columns. Commercial reagents of high purity were purchased and used without further purification unless otherwise noted. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AMX-400 instrument and calibrated to the residual solvent peak. The multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet).

The synthesis of 10-((2-pyridylsulfonyl)oxo)-*N*-biotinamidopentyldecanamide (1) is provided as a representative synthesis of the 11 biotinylated alkyl and aryl sulfonates (1–11).

#### 5.1.1. ((2-Pyridylsulfonyl)oxo)-10-undecene (13)

A solution of  $\omega$ -undecylenyl alcohol (12) (0.50 g, 2.91 mmol, 1.0 equivalents (equiv.)) in pyridine (4 ml) was cooled to 0°C and treated with 2-pyridylsulfonyl chloride (1.04 g, 5.87 mmol, 2.0 equiv.), prepared according to the procedure of Corey and colleagues [44]. The reaction mixture was kept at 0°C for 6 h, then partitioned between ethyl acetate (50 ml) and water (25 ml). The organic layer was washed with 10% aqueous HCl (2 × 50 ml) and saturated aqueous NaCl (50 ml), dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure. Column chromatography (2% EtOAc/Hex) afforded 13 as a colorless oil (98%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz),  $\delta$  8.61 (m, 1H, ArH), 7.89 (m, 2H, ArH), 7.47 (m, 1H, ArH), 5.67–5.60 (m, 1H,  $\text{RCH}=\text{CH}_2$ ), 4.84–4.74 (m, 2H,  $\text{RCH}=\text{CH}_2$ ), 4.21 (t,  $J$  = 6.4 Hz, 2H,  $\text{CH}_2\text{OSO}_2\text{Pyr}$ ), 1.88 (m, 2H,  $\text{CH}_2\text{CH}=\text{CH}_2$ ), 1.55 (p, 2H,  $J$  = 6.8 Hz, 2H,  $\text{CH}_2\text{CH}_2\text{OSO}_2\text{Pyr}$ ), 1.20–1.08 (m, 12H); MALDI-FTMS 334.1433 ( $\text{C}_{16}\text{H}_{25}\text{NO}_3\text{S}+\text{Na}^+$  requires 334.1447).

#### 5.1.2. 10-((2-Pyridylsulfonyl)oxo)-decanoic acid (14)

Compound 13 (0.90 g, 2.88 mmol, 1 equiv.) in a bipha-

sic solution composed of  $\text{CCl}_4$ – $\text{CH}_3\text{CN}$ – $\text{H}_2\text{O}$  (10 ml–10 ml–15 ml) with a total volume of 35 ml was treated sequentially with sodium periodate (2.53 g, 11.80 mmol, 4.1 equiv.) and ruthenium trichloride hydrate (0.005 g, 0.02 mmol, 0.03 equiv.). The reaction was stirred at 25°C overnight then partitioned between  $\text{CH}_2\text{Cl}_2$  (100 ml) and 1 N aqueous HCl (2 × 100 ml). The organic layer was washed with saturated aqueous NaCl (100 ml), dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. Column chromatography (40% EtOAc/Hex) afforded 14 (80%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz),  $\delta$  8.84 (d,  $J$  = 4.0 Hz, 1H, ArH), 8.11 (d,  $J$  = 5.9 Hz, 1H, ArH), 8.05 (t,  $J$  = 6.0 Hz, 1H, ArH), 7.65 (t,  $J$  = 3.3 Hz, 1H, ArH), 4.37 (t,  $J$  = 6.6 Hz, 2H,  $\text{CH}_2\text{OSO}_2\text{Pyr}$ ), 2.34 (t,  $J$  = 7.4 Hz, 2H,  $\text{CH}_2\text{COOH}$ ), 1.70 (p,  $J$  = 8.0 Hz, 2H,  $\text{CH}_2\text{CH}_2\text{COOH}$ ), 1.61 (p,  $J$  = 7.3 Hz, 2H,  $\text{CH}_2\text{CH}_2\text{OSO}_2\text{Pyr}$ ), 1.25 (m, 10H); MALDI-FTMS (DHB)  $m/z$  352.1202 ( $\text{C}_{15}\text{H}_{23}\text{NO}_5\text{S}+\text{Na}^+$  requires 352.1189).

#### 5.1.3. 10-((2-Pyridylsulfonyl)oxo)-*N*-biotinamidopentyldecanamide (1)

A solution of 14 (0.030 g, 0.09 mmol, 10 equiv.) in  $\text{CH}_2\text{Cl}_2$  (1.5 ml) at –78°C was treated dropwise with (diethylamino)sulfur trifluoride (0.027 ml, 0.21 mmol, 22 equiv.), brought to 25°C, and stirred for 10 min. The reaction was then treated with one-half reaction volume of dimethylformamide containing *N*-hydroxysuccinimide (0.05 g, 0.04 mmol, 40 equiv.) and stirred for an additional 15 min at 25°C. The reaction mixture was partitioned between ethyl acetate (50 ml) and water (50 ml). The organic layer was washed with saturated aqueous NaCl (200 ml), dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated under reduced pressure to afford 10-((2-pyridylsulfonyl)oxo)-*N*-(hydroxysuccinyl)decanamide (as judged by crude  $^1\text{H}$  NMR; data not shown). Without further purification, the intermediate was treated with 5-(biotinamido)-pentylamine (Pierce, 0.003 g, 0.009 mmol, 1.0 equiv.) in MeOH (0.04 ml) and stirred for 30 min. The solvent was evaporated under a stream of nitrogen, and the remaining residue was washed with ethyl acetate (2 × 2.5 ml), solubilized in a minimal volume of chloroform, transferred to a clean glass vial, and the solvent evaporated. This process was repeated to rid the desired biotinylated product of excess reagents and byproducts, affording 1 as a white film (0.004 g, 46%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz),  $\delta$  8.79 (d,  $J$  = 7.0 Hz, 1H, ArH), 8.06 (m, 2H, ArH), 7.61 (t,  $J$  = 7.3 Hz, 1H, ArH), 6.05 (b s, 1H, NH), 5.91 (b s, 1H, NH), 5.60 (b s, 1H, NH), 4.77 (b s, 1H, NH), 4.54 (m, 1H), 4.39 (m, 1H+2H,  $\text{CH}_2\text{OSO}_2\text{R}$ ), 3.22 (m, 4H,  $\text{CH}_2\text{NHCOR}$ ), 3.11 (m, 1H), 2.92 (dd,  $J$  = 4.0 and 12.9 Hz, 1H), 2.76 (d,  $J$  = 13.3 Hz, 1H), 2.18 (m, 4H,  $\text{CH}_2\text{CONHR}$ ), 1.67–1.28 (m, 26H); MALDI-FTMS (DHB)  $m/z$  640.3209 ( $\text{C}_{30}\text{H}_{49}\text{N}_5\text{O}_6\text{S}_2+\text{H}^+$  requires 640.3202).

## 5.1.4. 10-((Benzenesulfonyl)oxo)-N-biotinamidopentyldecanamide (2)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  7.93 (d,  $J$  = 7.0 Hz, 2H, ArH), 7.67 (t,  $J$  = 7.4 Hz, 1H, ArH), 7.59 (t,  $J$  = 7.3 Hz, 2H, ArH), 6.06 (b s, 1H, NH), 5.87 (b s, 1H, NH), 5.63 (b s, 1H, NH), 4.79 (b s, 1H, NH), 4.53 (m, 1H), 4.37 (m, 1H), 4.07 (t,  $J$  = 6.4 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.26 (m, 4H, CH<sub>2</sub>NHCOR), 3.09 (m, 1H), 2.93 (dd,  $J$  = 5.0 and 7.9 Hz, 1H), 2.76 (d,  $J$  = 13.0 Hz, 1H), 2.19 (m, 4H, CH<sub>2</sub>CONHR), 1.62-1.26 (m, 26H); MALDI-FTMS (DHB)  $m/z$  639.3244 (C<sub>31</sub>H<sub>50</sub>N<sub>4</sub>O<sub>6</sub>S<sub>2</sub>+H<sup>+</sup> requires 639.3245).

## 5.1.5. 10-((p-Toluenesulfonyl)oxo)-N-biotinamidopentyldecanamide (3)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  7.78 (d,  $J$  = 8.5 Hz, 2H, ArH), 7.37 (d,  $J$  = 7.9 Hz, 2H, ArH), 5.91 (b s, 1H, NH), 5.84 (b s, 1H, NH), 5.49 (b s, 1H, NH), 4.76 (b s, 1H, NH), 4.53 (m, 1H), 4.35 (m, 1H), 4.02 (t,  $J$  = 6.4 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.25 (m, 4H, CH<sub>2</sub>NHCOR), 3.18 (m, 1H), 2.93 (dd,  $J$  = 5.0 and 7.9 Hz, 1H), 2.76 (d,  $J$  = 12.9 Hz, 1H), 2.46 (s, 3H, CH<sub>3</sub>Ar), 2.19 (m, 4H, CH<sub>2</sub>CONHR), 1.70-1.50 (m, 26H); MALDI-FTMS (DHB)  $m/z$  653.3381 (C<sub>32</sub>H<sub>52</sub>N<sub>4</sub>O<sub>6</sub>S<sub>2</sub>+H<sup>+</sup> requires 653.3401).

## 5.1.6. 10-((4-Methoxybenzenesulfonyl)oxo)-N-biotinamidopentyldecanamide (4)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  7.86 (d,  $J$  = 8.8 Hz, 2H, ArH), 7.03 (d,  $J$  = 8.8 Hz, 2H, ArH), 5.96 (b s, 1H, NH), 5.85 (b s, 1H, NH), 5.57 (b s, 1H, NH), 4.84 (b s, 1H, NH), 4.53 (m, 1H), 4.37 (m, 1H), 4.01 (t,  $J$  = 6.5 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.90 (s, 3H, CH<sub>3</sub>OAr), 3.25 (m, 4H, CH<sub>2</sub>NHCOR), 3.17 (m, 1H), 2.95 (dd,  $J$  = 4.7 and 7.7 Hz, 1H), 2.73 (d,  $J$  = 12.9 Hz, 1H), 2.25 (m, 4H, CH<sub>2</sub>CONHR), 1.63-1.26 (m, 26H); MALDI-FTMS (DHB)  $m/z$  669.3381 (C<sub>32</sub>H<sub>52</sub>N<sub>4</sub>O<sub>7</sub>S<sub>2</sub>+H<sup>+</sup> requires 669.335).

## 5.1.7. 10-((Methylsulfonyl)oxo)-N-biotinamidopentyldecanamide (5)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  6.00 (b s, 1H, NH), 5.85 (b s, 1H, NH), 5.60 (b s, 1H, NH), 4.81 (b s, 1H, NH), 4.53 (m, 1H), 4.37 (m, 1H), 4.35 (t,  $J$  = 6.2 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.26 (m, 4H, CH<sub>2</sub>NHCOR), 3.18 (m, 1H), 2.93 (dd,  $J$  = 5.0 and 7.9 Hz, 1H), 2.76 (d,  $J$  = 12.9 Hz, 1H), 2.21 (m, 4H, CH<sub>2</sub>CONHR), 2.05 (s, 3H, H<sub>3</sub>CSO<sub>3</sub>R), 1.75-1.27 (m, 26H); MALDI-FTMS  $m/z$  577.3105 (C<sub>26</sub>H<sub>48</sub>N<sub>4</sub>O<sub>6</sub>S<sub>3</sub>+H<sup>+</sup> requires 577.3088).

## 5.1.8. 10-((Butylsulfonyl)oxo)-N-biotinamidopentyldecanamide (6)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  5.93 (b s, 1H, NH), 5.84 (b s, 1H, NH), 5.53 (b s, 1H, NH), 4.82 (b s, 1H, NH), 4.54 (m, 1H), 4.37 (m, 1H), 4.21 (t,  $J$  = 6.2 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.26 (m, 4H, CH<sub>2</sub>NHCOR), 3.19 (m, 1H), 3.09 (t,  $J$  = 4.1 Hz, 2H, CH<sub>2</sub>SO<sub>3</sub>R), 2.93 (dd,  $J$  = 4.6 and

7.4 Hz, 1H), 2.76 (d,  $J$  = 9.1 Hz, 1H), 2.21 (m, 4H, CH<sub>2</sub>CONHR), 2.16-1.31 (m, 30H), 0.97 (t,  $J$  = 7.3 Hz, 3H); MALDI-FTMS (DHB)  $m/z$  619.3530 (C<sub>29</sub>H<sub>54</sub>N<sub>4</sub>O<sub>6</sub>S<sub>2</sub>+H<sup>+</sup> requires 619.3530).

## 5.1.9. 10-((Octylsulfonyl)oxo)-N-biotinamidopentyldecanamide (7)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  6.05 (b s, 1H, NH), 5.87 (b s, 1H, NH), 5.81 (b s, 1H, NH), 5.00 (b s, 1H, NH), 4.53 (m, 1H), 4.35 (m, 1H), 4.21 (t,  $J$  = 6.2 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.25 (m, 4H, CH<sub>2</sub>NHCOR), 3.17 (m, 1H), 3.09 (t,  $J$  = 8.5 Hz, 2H, CH<sub>2</sub>SO<sub>3</sub>R), 2.93 (dd,  $J$  = 4.7 and 7.9 Hz, 1H), 2.76 (d,  $J$  = 12.9 Hz, 1H), 2.19 (m, 4H, CH<sub>2</sub>CONHR), 1.86 (p,  $J$  = 7.9 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>SO<sub>3</sub>R), 1.76-1.31 (m, 36H), 0.89 (t,  $J$  = 6.4 Hz, 3H); MALDI-FTMS (DHB)  $m/z$  675.4173 (C<sub>33</sub>H<sub>62</sub>N<sub>4</sub>O<sub>6</sub>S<sub>2</sub>+H<sup>+</sup> requires 675.4184).

## 5.1.10. 10-((4-Nitrobenzenesulfonyl)oxo)-N-biotinamidopentyldecanamide (8)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  8.44 (d,  $J$  = 9.1 Hz, 2H, ArH), 8.14 (d,  $J$  = 9.1 Hz, 2H, ArH), 5.90 (b s, 1H, NH), 5.82 (b s, 1H, NH), 5.50 (b s, 1H, NH), 4.86 (b s, 1H, NH), 4.54 (m, 1H), 4.37 (m, 1H), 4.14 (t,  $J$  = 6.5 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.25 (m, 4H, CH<sub>2</sub>NHCOR), 3.18 (m, 1H), 2.96 (dd,  $J$  = 5.0 and 7.9 Hz, 1H), 2.76 (d,  $J$  = 15.0 Hz, 1H), 2.21 (m, 4H, CH<sub>2</sub>CONHR), 1.72-1.26 (m, 26H); MALDI-FTMS (DHB)  $m/z$  684.3069 (C<sub>31</sub>H<sub>49</sub>N<sub>5</sub>O<sub>8</sub>S<sub>2</sub>+H<sup>+</sup> requires 684.3095).

## 5.1.11. 10-((8-Quinolinesulfonyl)oxo)-N-biotinamidopentyldecanamide (9)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  9.17 (m, 1H, ArH), 8.53 (d,  $J$  = 5.8 Hz, 1H, ArH), 8.50 (d,  $J$  = 6.4 Hz, 1H, ArH), 8.16 (d,  $J$  = 6.7 Hz, 1H, ArH), 7.70 (t,  $J$  = 7.6 Hz, 1H, ArH), 7.61 (q,  $J$  = 3.9 Hz, 1H, ArH), 6.06 (b s, 1H, NH), 5.93 (b s, 1H, NH), 5.73 (b s, 1H, NH), 5.08 (b s, 1H, NH), 4.54 (m, 1H), 4.35 (m, 1H), 4.30 (t,  $J$  = 6.5 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.27 (m, 4H, CH<sub>2</sub>NHCOR), 3.18 (m, 1H), 2.96 (dd,  $J$  = 5.0 and 7.9 Hz, 1H), 2.77 (d,  $J$  = 15 Hz, 1H), 2.21 (m, 4H, CH<sub>2</sub>CONHR), 1.68-1.18 (m, 26H); MALDI-FTMS 712.3189 (C<sub>34</sub>H<sub>51</sub>N<sub>5</sub>O<sub>6</sub>S<sub>2</sub>+Na<sup>-</sup> requires 712.3173).

## 5.1.12. 10-((2-Naphthalenesulfonyl)oxo)-N-biotinamidopentyldecanamide (10)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz),  $\delta$  8.49 (s, 1H, ArH), 7.98 (t,  $J$  = 8.2 Hz, 2H, ArH), 7.93 (d,  $J$  = 7.9 Hz, 1H, ArH), 7.87 (d,  $J$  = 8.8 Hz, 1H, ArH), 7.65 (p,  $J$  = 7.0 Hz, 2H, ArH), 6.05 (b s, 1H, NH), 5.91 (b s, 1H, NH), 5.60 (b s, 1H, NH), 4.77 (b s, 1H, NH), 4.53 (m, 1H), 4.37 (m, 1H), 4.06 (t,  $J$  = 6.4 Hz, 2H, CH<sub>2</sub>OSO<sub>2</sub>R), 3.25 (m, 4H, CH<sub>2</sub>CONHR), 3.17 (m, 1H), 2.95 (dd,  $J$  = 5.0 and 7.6 Hz, 1H), 2.77 (d,  $J$  = 14.0 Hz), 2.17 (m, 4H, CH<sub>2</sub>CONHR), 1.71-1.20 (m, 26H); MALDI-FTMS

(DHB)  $m/z$  689.3379 ( $C_{35}H_{52}N_4O_6S_2+H^+$  requires 689.3401).

#### 5.1.13. 10-((2-Thiophenesulfonyl)oxo)-N-biotinamidopentyl)decanamide (11)

$^1H$  NMR ( $CDCl_3$ , 400 MHz),  $\delta$  7.74 (t,  $J=5.0$  Hz, 2H, ArH), 7.16 (t,  $J=3.8$  Hz, 1H, ArH), 5.90 (b s, 1H, NH), 5.84 (b s, 1H, NH), 5.47 (b s, 1H, NH), 4.75 (b s, 1H, NH), 4.53 (m, 1H), 4.35 (m, 1H), 4.12 (t,  $J=6.4$  Hz, 2H,  $CH_2OSO_2R$ ), 3.26 (m, 4H,  $CH_2NHCOR$ ), 3.19 (m, 1H), 2.95 (dd,  $J=5.3$  and 7.6 Hz, 1H), 2.76 (d,  $J=12.9$  Hz, 1H), 2.17 (m, 4H,  $CH_2CONHR$ ), 1.67–1.26 (m, 26H); MALDI-FTMS (DHB)  $m/z$  645.2817 ( $C_{29}H_{48}N_4O_6S_3+H^+$  requires 645.2809).

#### 5.1.14. 1-(2-Pyridylsulfonyl)oxo-octane (15)

To 3.0 ml of anhydrous triethylamine (23.04 mmol, 30 equiv.) at 0°C was added 1-octanol (0.10 g, 0.77 mmol, 1 equiv.) followed by the addition of 2-pyridylsulfonyl chloride in one portion. The mixture was kept at 0°C for 3 h followed by the addition of water (5 ml). The resulting mixture was extracted with diethyl ether (3 × 50 ml), then the organic extracts were combined and washed with aqueous  $NaHCO_3$  solution (50 ml), dried ( $MgSO_4$ ), and concentrated under reduced pressure. Column chromatography (2% EtOAc/Hex) afforded 15 (99%):  $^1H$  NMR ( $CDCl_3$ , 400 MHz),  $\delta$  8.68 (d,  $J=7.0$  Hz, 1H, ArH), 7.94 (p,  $J=8.8$  Hz, 2H, ArH), 7.54 (t,  $J=6.4$  Hz, 1H, ArH), 4.26 (t,  $J=6.7$  Hz, 2H,  $CH_2OSO_2R$ ), 1.61 (p,  $J=7.9$  Hz, 2H), 1.13 (m, 10H), 0.76 (t,  $J=7.0$  Hz, 3H); MALDI-FTMS 294.1130 ( $C_{13}H_{21}NO_3S+Na^+$  requires 294.1134).

#### 5.1.15. 1-(2-Pyridylsulfonyl)oxo-ethane (16)

To a solution of triethylamine (0.86 g, 8.51 mmol, 2.2 equiv.) in dichloromethane (3 ml) at 0°C was added ethanol (0.18 g, 3.87 mmol, 1 equiv.) followed by the addition of 2-pyridylsulfonyl chloride (0.83 g, 4.65 mmol, 1.2 equiv.). After stirring for 4 h at 0°C, the solution was concentrated under reduced pressure. The concentrate was dissolved in aqueous  $NaHCO_3$  solution (50 ml) and extracted with diethyl ether (3 × 50 ml). The ether extracts were combined and washed with aqueous NaCl (50 ml), dried ( $MgSO_4$ ), and concentrated under reduced pressure. Column chromatography (5% EtOAc/Hex) afforded 16 (95%):  $^1H$  NMR ( $CDCl_3$ , 400 MHz),  $\delta$  8.70 (d,  $J=4.7$  Hz, 1H, ArH), 7.95 (p,  $J=6.4$  Hz, 2H, ArH), 7.56 (t,  $J=5.3$  Hz, 1H, ArH), 4.37 (q,  $J=7.0$  Hz, 2H,  $CH_2OSO_2R$ ), 1.29 (t,  $J=7.0$  Hz, 3H); MALDI-FTMS 188.0000 ( $C_6H_9NOS+H^+$  requires 188.0376).

#### 5.1.16. 1-(Methanesulfonyl)oxo-octane (17)

To a solution of triethylamine (0.12 g, 1.15 mmol, 1.5 equiv.) in dichloromethane (3 ml) was added octanol (0.10 g, 0.77 mmol, 1.0 equiv.) at 0°C followed by the addition of methanesulfonyl chloride (0.10 g, 0.85 mmol, 1.1

equiv.), over a period of 5 min. After 30 min at 0°C, the reaction mixture was diluted in dichloromethane (50 ml) and extracted with ice cold water (50 ml), ice cold 10% aqueous HCl (50 ml), saturated aqueous  $NaHCO_3$  (50 ml), and with saturated aqueous NaCl (50 ml). The organic layer was dried ( $MgSO_4$ ) and concentrated under reduced pressure to afford 17 (97%):  $^1H$  NMR ( $CDCl_3$ , 400 MHz),  $\delta$  4.21 (t,  $J=6.5$  Hz, 2H,  $CH_2OSO_2Me$ ), 2.99 (s, 3H,  $CH_3SO_3$ ), 1.77 (p,  $J=6.7$  Hz, 2H), 1.35 (m, 10 H), 0.87 (t,  $J=6.1$  Hz, 3H).

#### 5.2. Proteome sample preparation, labeling, and detection

Rat tissues were Dounce-homogenized in Tris buffer (50 mM Tris-HCl buffer, pH 8.0, 0.32 M sucrose). Tissue extracts were centrifuged sequentially at 1100 ×  $g$  (10 min), 22000 ×  $g$  (30 min), and 105000 ×  $g$  (60 min). The final supernatant (soluble fraction) was adjusted to 0.5 mg protein/ml with Tris buffer (without sucrose) and kept at 0°C until utilized. Unless otherwise indicated, reactions between protein samples and biotinylated reagents were conducted as follows: all biotinylated reagents were stored as stock solutions in DMSO at -20°C and then added directly to reactions with protein extracts, keeping the DMSO concentration constant at 1% of the total reaction volume. The reaction mixture was incubated at 25°C for 30 min (final concentration of the probe was 5  $\mu M$ ), then quenched by the addition of 1 volume equiv. of standard 2 × SDS-PAGE loading buffer (reducing). Quenched reactions were run on SDS-PAGE (7.5  $\mu g$  protein/gel lane) and transferred by electroblotting onto nitrocellulose membranes, which were blocked in Tris-buffered saline (TBS) with 1% Tween (TBS-Tween) and 3% (w/v) non-fat dry milk for either 1 h at 25°C or overnight at 4°C. Blots were then treated with an avidin-horseradish peroxidase conjugate (Bio-Rad, 1:1500 dilution) in TBS-Tween for 2 h at 25°C. The blot was washed with TBS-Tween three times (5 min/wash), treated with SuperSignal chemiluminescence reagents (Bio-Rad), and exposed to film for 0.1–20 min before development. For the pH-dependence studies, the following reaction buffers were used: pH 6–8: 50 mM Tris-HCl; pH 8–10: 50 mM Tris-HCl, 50 mM CAPS.

#### 5.3. Enrichment and molecular characterization of a 55 kDa sulfonate-reactive protein

Rat liver soluble fractions were run over a Q-Sepharose column by using an AKTA FPLC (Amersham Pharmacia Biotech) and eluted with a linear gradient of 0–500 mM NaCl. Aliquots of the elution fractions (10 × 2.5 ml fractions) as well as the flow-through (3 × 2.5 ml fractions) were labeled with 1 as described above to identify the fractions containing the labeled proteins. The flow-through fractions, which contained the 55 kDa protein, were concentrated to 1 mg protein/ml followed by labeling

2.5 ml of the sample with 1 utilizing the standard conditions. After incubating the reaction for 30 min, it was applied to a PD-10 size exclusion column and eluted with 3.5 ml of pH 8, 50 mM Tris-HCl buffer. SDS (0.5% w/v) was added and the labeled samples heated to 90°C for 10 min in order to denature the proteins allowing for a more accessible biotin moiety. The sample was then diluted 2.5-fold (0.2% SDS) and incubated with 50-100  $\mu$ l of avidin beads on a rotator for 1 h at 25°C. The eluant was then removed followed by washing with 5 ml of 0.2% SDS and three washes with pH 8, 50 mM Tris-HCl buffer. Standard 2 $\times$  SDS-PAGE loading buffer was added followed by heating the sample to 90°C in order to elute the proteins labeled with 1 from the avidin beads. The eluant was run on an 8% Novex Tris-glycine gel and stained with Coomassie blue stain followed by destaining in a 30% methanol-water solution. The desired 55 kDa 1-reactive protein was excised from the gel and digested with trypsin. The resulting peptides were analyzed by MALDI-TOF MS. The MALDI peptide data were utilized in both the MS-Fit search of the Protein Prospector databases ([falcon.ludwig.ucl.ac.uk/mskome3.2.htm](http://falcon.ludwig.ucl.ac.uk/mskome3.2.htm)) and the ProFound search of the Proteometrics databases ([www.proteometrics.com/prowl-cgi/ProFound.exe](http://www.proteometrics.com/prowl-cgi/ProFound.exe)), which identified the protein as cALDH-I.

#### 5.4. Recombinant expression and purification of cALDH-I

Primers were designed based on cALDH-I's cDNA sequence and used to amplify the enzyme's cDNA from a liver cDNA library (Clontech). The cALDH-I cDNA was subcloned into the prokaryotic expression vector, TrcHisA, followed by transformation and expression in *E. coli* BL-21 cells. Expression was induced with 1 mM isopropyl  $\beta$ -D-thiogalactoside when cultures grew to an OD<sub>600</sub> of 0.6. After 4 h, the cells were pelleted and the supernatant removed. The cell pellet was resuspended in Tris buffer (20 mM Tris-HCl buffer, pH 8.0, 100 mM NaCl), lysed by treatment with lysozyme (1 mg/ml) for 30 min and then sonication. The soluble fraction was isolated by centrifugation 39 800 $\times$ g (25 min). The His-tagged cALDH-I was purified from the soluble fraction by rotating with Talon cobalt beads for 30 min at 4°C followed by centrifugation and removal of the lysate. After washing, the beads were eluted with 80 mM imidazole buffer and the eluted protein concentrated to 10 mg protein/ml. The concentrated protein solution was subjected to gel filtration chromatography (Superose 6 column, AKTA FPLC, Amersham Pharmacia Biotech). Gel filtration samples containing purified cALDH-I were combined, concentrated, and stored at -78°C in Tris buffer containing 1 mM DTT (final cALDH-I protein concentration, 1.5 mg/ml).

#### 5.5. Expression of cALDH-I in eukaryotic cells

The cALDH-I cDNA was subcloned into the eukaryotic

expression vector pcDNA3 and transiently transfected into COS-7 cells and MCF-7 cells by using methods described previously [14]. Transfected cells were harvested by scraping, resuspended in Tris buffer and their protein concentrations determined (D<sub>c</sub> protein assay kit, Bio-Rad). Whole cell suspensions were labeled with the sulfonate 1 as described above.

#### 5.6. Characterization of sulfonate reactivity of cALDH-I

cALDH-I-transfected COS-7 cells were harvested by scraping, resuspended in Tris buffer, Dounce-homogenized, and sonicated. The soluble fraction was separated by centrifugation and the protein concentration determined (D<sub>c</sub> protein assay kit, Bio-Rad). The soluble lysate (0.5  $\mu$ g/ $\mu$ l) was incubated with each biotinylated probe (2.5  $\mu$ M, 1% DMSO) for 2 min at 25°C in Tris buffer (50 mM Tris-HCl, pH 8). A mock-transfected COS-7 sample was labeled with sulfonate 1 as a control. The reactions were quenched and analyzed by SDS-PAGE and avidin blotting as described above.

#### 5.7. cALDH-I enzyme assay and inhibition studies

cALDH-I activity was determined at 25°C in Tris buffer (20 mM Tris-HCl, pH 8, 100 mM NaCl). Purified cALDH-I (0.2  $\mu$ M) was preincubated with 15 (2.5-15  $\mu$ M) in DMSO (30  $\mu$ l, 3% total incubation volume) in a volume of 950  $\mu$ l for 5-45 min. After preincubation of the enzyme with inhibitor, remaining catalytic activity was measured by adding NAD<sup>+</sup> (500  $\mu$ M final concentration) and propionaldehyde (1 mM final concentration) in 50  $\mu$ l of buffer. Production of NADH from the oxidation of propionaldehyde was monitored by measuring the change in absorbance at 340 nm for 2 min. In substrate competition assays, purified cALDH-I (0.2  $\mu$ M) was preincubated with either NAD<sup>+</sup> (50  $\mu$ M) or propionaldehyde (25  $\mu$ M) and 10  $\mu$ M of 15 for 10 min at 25°C in a volume of 950  $\mu$ l. Remaining catalytic activity was monitored as described above.

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## Printing Proteins as Microarrays for High-Throughput Function Determination

Gavin MacBeath<sup>1\*</sup> and Stuart L. Schreiber<sup>2</sup>

as single-nucleotide polymorphism analysis, where single-mismatch resolution, sensitivity, cost, and ease of use are important factors. Moreover, the sensitivity of this system, which has yet to be totally optimized, points toward a potential method for detecting oligonucleotide targets without the need for target amplification schemes such as the polymerase chain reaction.

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10. For the experiments reported in Fig. 2, dissociation measurements were made from the surface of glass beads 250 to 300  $\mu\text{m}$  in diameter (Polysciences, Warrington, PA) rather than planar substrates to increase the UV-visible and fluorescence signal intensity.
11. 5'-Cy3-labeled oligonucleotide probes were synthesized on an Expedite automated synthesizer (Millipore, Bedford, MA) using Cy3 phosphoramidite (Glen Research, Sterling, VA) as the label source. Arrays of spots 175  $\mu\text{m}$  in diameter separated by 375  $\mu\text{m}$  were patterned with a GMS 417 Microarrayer (Genetic Microsystems, Woburn, MA).
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14. First, 20  $\mu\text{l}$  of a 1 mM solution of synthetic target in 2 $\times$  phosphate-buffered saline (PBS) [0.3 M NaCl and 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 7)] was hybridized to the array for 4 hours at 10°C in a CoverWell PC20 hybridization chamber (Grace Bio-Labs, Bend, OR) and was then washed at 10°C with clean PBS buffer. Next, 20  $\mu\text{l}$  of a 100 pM solution of either oligonucleotide-functionalized gold nanoparticles or 5'-Cy3-labeled probe in 2 $\times$  PBS was hybridized to the array for 4 hours at 10°C in a fresh hybridization chamber. The array was then washed at the stringency temperature (shown in Fig. 3) with clean 2 $\times$  PBS buffer for 2 min. Arrays labeled with nanoparticle probes were washed twice at room temperature with 2 $\times$  PBN [0.3 M  $\text{NaNO}_3$  and 10 mM  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer (pH 7)], then submerged in Silver Enhancer Solution (Sigma) for 5 min at room temperature and washed with water.
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16. The temperature ranges for the melting curves in Fig. 2 do not correspond exactly with the stringency temperatures associated with the oligonucleotide array experiments reported in Fig. 3. This is probably because the two sets of experiments are not identical with respect to the substrate.
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18. C.A.M. and R.L.L. acknowledge the Army Research Office (DAAG55-97-1-0133) and the National Institute of General Medical Sciences (GM 57356) for support of this work.

Systematic efforts are currently under way to construct defined sets of cloned genes for high-throughput expression and purification of recombinant proteins. To facilitate subsequent studies of protein function, we have developed miniaturized assays that accommodate extremely low sample volumes and enable the rapid, simultaneous processing of thousands of proteins. A high-precision robot designed to manufacture complementary DNA microarrays was used to spot proteins onto chemically derivatized glass slides at extremely high spatial densities. The proteins attached covalently to the slide surface yet retained their ability to interact specifically with other proteins, or with small molecules, in solution. Three applications for protein microarrays were demonstrated: screening for protein-protein interactions, identifying the substrates of protein kinases, and identifying the protein targets of small molecules.

Historically, genome-wide screens for protein function have been carried out with random cDNA libraries. Most frequently, the libraries are prepared in phage vectors and the expressed proteins immobilized on a membrane by a plaque lift procedure. This method has been effective for a variety of applications (1–4), but it has several limitations. Most clones in the library do not encode proteins in the correct reading frame, and most proteins are not full-length. Bacterial expression of eukaryotic genes frequently fails to yield correctly folded proteins, and products derived from abundant transcripts are overrepresented. Moreover, because plaque lifts are not amenable to miniaturization on the micrometer scale, it is hard to imagine screening all the proteins of an organism hundreds or thousands of times by this approach.

With the advent of high-throughput molecular biology, it is now possible to prepare large, normalized collections of cloned genes. UniGene sets in the form of polymerase chain reaction products have been used extensively over the past decade to construct DNA microarrays for the study of transcriptional regulation (5). Recently, spatially segregated clones in expression vectors were used to study protein function *in vivo* using the yeast two-hybrid system (6) and *in vitro* using biochemical assays (7). We have built on these efforts by developing microarray-based methods to study protein function.

To accomplish these goals, it is necessary to immobilize proteins on a solid support in a way that preserves their folded conformations. One

group has described methods of arraying functionally active proteins, using microfabricated polyacrylamide gel pads to capture their samples and microelectrophoresis to accelerate diffusion (8). In contrast, we have immobilized proteins by covalently attaching them to the smooth, flat surface of glass microscope slides. One of our primary objectives in pursuing this approach was to make the technology easily accessible and compatible with standard instrumentation. We use a variety of chemically derivatized slides that can be printed and imaged by commercially available arrayers and scanners. For most applications, we use slides that have been treated with an aldehyde-containing silane reagent (9). The aldehydes react readily with primary amines on the proteins to form a Schiff's base linkage. Because typical proteins display many lysines on their surfaces as well as the generally more reactive  $\alpha$ -amine at their  $\text{NH}_2$ -termini, they can attach to the slide in a variety of orientations, permitting different sides of the protein to interact with other proteins or small molecules in solution.

To fabricate protein microarrays, we use a high-precision contact-printing robot (10) to deliver nanoliter volumes of protein samples to the slides, yielding spots about 150 to 200  $\mu\text{m}$  in diameter (1600 spots per square centimeter). The proteins are printed in phosphate-buffered saline with 40% glycerol included to prevent evaporation of the nanodroplets. It is important that the proteins remain hydrated throughout this and subsequent steps to prevent denaturation. After a 3-hour incubation, the slides are immersed in a buffer containing bovine serum albumin (BSA). This step not only quenches the unreacted aldehydes on the slide, but also forms a molecular layer of BSA that reduces nonspecific binding of other proteins in subsequent steps.

Although appropriate for most applications, aldehyde slides cannot be used when peptides

<sup>1</sup>Center for Genomics Research, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA.

<sup>2</sup>Howard Hughes Medical Institute (HHMI), Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA.

\*To whom correspondence should be addressed. E-mail: [gavin\\_macbeath@harvard.edu](mailto:gavin_macbeath@harvard.edu)

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or very small proteins are printed, presumably because the BSA obscures the molecules of interest. For such applications, we use BSA-NHS (BSA-*N*-hydroxysuccinimide) slides that are fabricated by first attaching a molecular layer of BSA to the surface of glass slides and

then activating the BSA with *N,N'*-disuccinimidyl carbonate (11). The activated lysine, aspartate, and glutamate residues on the BSA react readily with surface amines on the printed proteins to form covalent urea or amide linkages. The slides are then quenched with glycine. In contrast to the aldehyde slides, proteins or peptides printed on BSA-NHS slides are displayed on top of the BSA monolayer, rendering them accessible to macromolecules in solution.

As a first application of protein microarrays, we have looked at protein-protein interactions. Until now, only the yeast two-hybrid system has been used to investigate such interactions systematically on a genome-wide scale (6). This *in vivo* method, although easy to implement and of great utility, has several limitations. Proteins that function as transcriptional activators yield false positives when expressed as DNA binding domain fusions. False negatives are encountered when proteins are displayed inappropriately or when the DNA binding domain fusions are produced in excess. Proteins that do not fold correctly in yeast are inaccessible, and posttranslational modifications (such as phosphorylation or glycosylation) cannot be controlled. Finally, it is impossible to control the environment (e.g., ion concentration, presence or absence of cofactors, temperature) during the experiment.

To determine whether microarrays could be used for these types of studies, we selected three pairs of proteins that are known to interact: protein G and immunoglobulin G (IgG) (12); p50 (of the nuclear factor NF- $\kappa$ B complex) and the NF- $\kappa$ B inhibitor I $\kappa$ B $\alpha$  (13); and the FKBP12-rapamycin binding (FRB) domain of FKBP-rapamycin-associated protein (FRAP) and the human immunophilin FKBP12 (12 kD FK506-binding protein) (14). The first two interactions occur without special requirements, whereas the third interaction depends on the presence of the small molecule rapamycin (14). We arrayed the first protein of each pair in quadruplicate on five aldehyde slides and probed each slide with a different fluorescently labeled protein (11).

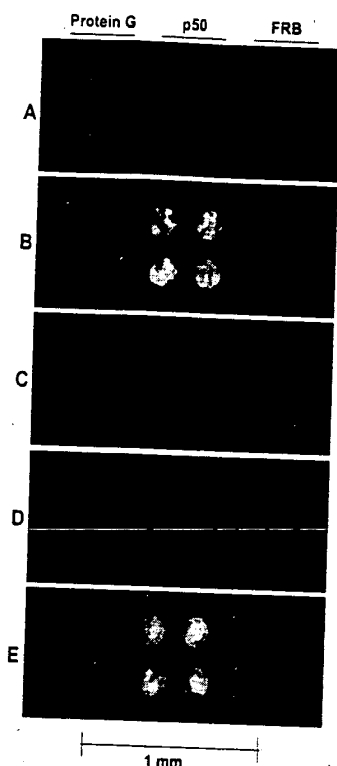
The slide in Fig. 1A was probed with BODIPY-FL-conjugated IgG, washed, and

scanned with an ArrayWoRx fluorescence slide scanner (15). As anticipated, only the spots containing protein G were visible, indicating that the immobilized protein is able to retain its functional properties on the glass surface. Similarly, only the p50-containing spots were visible on the slide probed with Cy3-I $\kappa$ B $\alpha$  (Fig. 1B) (15). For Cy5-FKBP12, binding to FRB was observed only when rapamycin was added (Fig. 1, C and D). Because the three fluorophores used for these studies have nonoverlapping excitation and emission spectra, we were also able to detect these interactions simultaneously (Fig. 1E).

By varying the concentration of FRB (the protein being immobilized), we found that at concentrations above 1 mg/ml, the fluorescence of the spots began to saturate. Below this, fluorescence scaled linearly with decreasing concentrations of FRB. All proteins immobilized on the slides described here were spotted at 100  $\mu$ g/ml. Because only a few microliters of each protein are sufficient to fabricate thousands of microarrays, purified proteins may be readily obtained by high-throughput expression and purification, or even by *in vitro* transcription/translation (16).

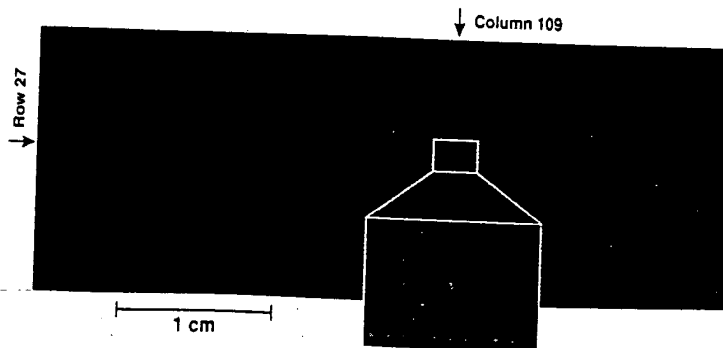
Much lower concentrations are needed for the solution-phase protein. In the case of Cy5-FKBP12, fluorescence scaled linearly with protein concentration over four orders of magnitude (11). Specific binding could be detected using Cy5-FKBP12 concentrations as low as 150 pg/ml ( $\sim$ 12.5 pM). Concentrations in this range are accessible not only with purified proteins, but also with fluorescently labeled proteins from cell lysates. Thus, specific interactions, once defined, may potentially be exploited to quantify protein abundance and modification in whole cells or tissues.

At the spot density used for these studies, it was possible to fit more than 10,000 samples in about half the area of a standard (2.5 cm by 7.5 cm) slide. To investigate the feasibility of detecting a single specific interaction in this larger context, we prepared a slide containing 60 rows and 180 columns of spatially separated spots. Protein G was spotted 10,799



**Fig. 1.** Detecting protein-protein interactions on glass slides. (A) Slide probed with BODIPY-FL-IgG (0.5  $\mu$ g/ml). (B) Slide probed with Cy3-I $\kappa$ B $\alpha$  (0.1  $\mu$ g/ml). (C) Slide probed with Cy5-FKBP12 (0.5  $\mu$ g/ml) and 100 nM rapamycin. (D) Slide probed with Cy5-FKBP12 (0.5  $\mu$ g/ml) and no rapamycin. (E) Slide probed with BODIPY-FL-IgG (0.5  $\mu$ g/ml), Cy3-I $\kappa$ B $\alpha$  (0.1  $\mu$ g/ml), Cy5-FKBP12 (0.5  $\mu$ g/ml), and 100 nM rapamycin. In all panels, BODIPY-FL, Cy3, and Cy5 fluorescence were false-colored blue, green, and red, respectively.

**Fig. 2.** A single slide holding 10,800 spots. Protein G was printed 10,799 times. A single spot of FRB was printed in row 27, column 109. The slide was probed with BODIPY-FL-IgG (0.5  $\mu$ g/ml), Cy5-FKBP12 (0.5  $\mu$ g/ml), and 100 nM rapamycin. BODIPY-FL and Cy5 fluorescence were false-colored blue and red, respectively.




times on this slide, with a single spot of FRB in row 27, column 109. The slide was then probed with a mixture of BODIPY-FL-IgG and Cy5-FKBP12, with 100 nM rapamycin included in the buffer. Figure 2 shows the single FRB spot, clearly visible in the sea of protein G spots.

Although isotopic labeling of the protein spots is the most direct way to identify phosphorylation, the challenge lies in detecting the radioactive decay. Neither x-ray film nor conventional PhosphorImagers offer sufficient spatial resolution to visualize the spots, which are 150 to 200  $\mu\text{m}$  in diameter. Borrowing from the technique of isotopic in situ hybridization, we dipped the slides in a photographic emulsion and developed them manually; this resulted in the deposition of silver grains directly on the glass surface. The slides


As the third and most demanding application, we sought to use protein microarrays to identify protein-small molecule interactions. With the advent of high-throughput, cell-based screening, more and more compounds are being identified on the basis of their biological activity. Once a "hit" is obtained, the daunting task of target identification remains. Several innovative techniques have been developed to address this bottleneck (4, 21-23), but they all suffer from the common limitations imposed by using random cDNA libraries. As an alternative, we sought to develop microarray-based assays that use purified, full-length, correctly folded proteins.

To investigate our ability to detect low-affinity interactions, we prepared Cy3-BSA conjugates of compounds API497, API1767, and API1780 (Fig. 4; dissociation constants for FKBP12 of 8.8 nM, 140 nM, and 2.6  $\mu$ M, respectively). When three identical slides displaying FKBP12 were probed in parallel, spots with comparable fluorescence intensities were obtained for all three conjugates


**AP1497**

R = 

**AP1767**

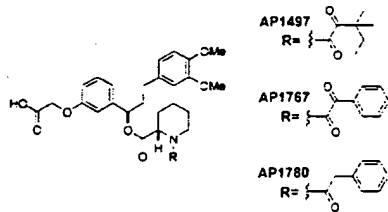
R = 

**AP1780**

R = 

(11). This means that interactions in the micromolar range can easily be observed. The fact that the intensity of the fluorescence did not vary appreciably as the affinity of the interaction was lowered can be attributed to the multivalency of the BSA conjugates (avidity effects). In the context of small-molecule microarrays (27), we have previously shown that when these three compounds are immobilized on a glass surface and then probed with Cy5-labeled FKBP12 (a monomeric protein), the intensity of the fluorescence correlates very well with the affinity of the interaction. Thus, by controlling the valency of the probe, we can choose whether to observe differences in affinity or to favor the detection of low-affinity interactions. The combination of these two approaches may prove useful in the identification of both primary and secondary drug targets.

Although traditional biochemical methods have yielded invaluable insight into protein function on a case-by-case basis, they cannot realistically be applied to the study of every protein in a cell, tissue, or organism. If we hope to assign function on a broader level, we must turn to miniaturized assays that can be performed in a highly parallel format. It is certainly a daunting task to express and purify thousands of different proteins, and some

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# REPORTS

## The Global Spread of Malaria in a Future, Warmer World

David J. Rogers<sup>1\*</sup> and Sarah E. Randolph<sup>2</sup>

proteins will inevitably prove refractory to biochemical manipulation. Nonetheless, the effort will be worthwhile if the many proteins that are amenable can be assayed both simultaneously and repeatedly. By fabricating protein microarrays, we can fulfill both these criteria, facilitating the in vitro study of protein function on a genome-wide level.

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The frequent warnings that global climate change will allow *falciparum* malaria to spread into northern latitudes, including Europe and large parts of the United States, are based on biological transmission models driven principally by temperature. These models were assessed for their value in predicting present, and therefore future, malaria distribution. In an alternative statistical approach, the recorded present-day global distribution of *falciparum* malaria was used to establish the current multivariate climatic constraints. These results were applied to future climate scenarios to predict future distributions, which showed remarkably few changes, even under the most extreme scenarios.

Predictions of global climate change have stimulated forecasts that vector-borne diseases will spread into regions that are at present too cool for their persistence (1–5). For example, life-threatening cerebral malaria, caused by *Plasmodium falciparum* transmitted by anopheline mosquitoes, is predicted to reach the central or northern regions of Europe and large parts of North America (2, 4). *falciparum* malaria is the most severe form of the human disease, causing most of the ~1 million deaths worldwide among the ~273 million cases in 1998 (6). Despite these figures, the epidemiology of malaria, like many other vector-borne tropical diseases, remains inadequately understood. Only the most general of maps for its worldwide distribution are available (7), and its global transmission patterns cannot be modeled satisfactorily because crucial parameters and their relations with environmental factors have not yet been quantified. Most importantly, absolute mosquito abundance has not yet been related to multivariate climate.

Nevertheless, the problem of malaria has led to its being included in most predictions about the impact of climate change on the future distribution of vector-borne diseases (8). These studies, which draw on the forecasts of future climate from various global circulation models (GCMs) (9, 10), generally use only one or at most two climatic variables to make their predictions. Biological models for malaria distribution are based principally on the temperature dependence of mosquito blood-feeding intervals and longevity and the development period of the malaria parasite within the mosquito, each of which affects the rate of transmission (4, 11). Those models based on threshold values include a lower temperature threshold, below which all development of the malaria parasite ceases, and an upper limit of mosquito

lethality (2). In addition, the suitability (or unsuitability) of habitats for these vectors, which require a minimum atmospheric moisture, is defined by the ratio of rainfall to potential evapotranspiration (2). The output of such models, therefore, represents predicted areas where parasite development within the vector is fast enough to be completed before the vector dies, bounded by limits imposed by habitat suitability (2). The fit of these predictions to the current global malaria situation shows noticeable mismatches in certain places (12); false predictions of presence (e.g., over the eastern half of the United States) are accounted for by past control measures or by "peculiar vector biogeography," whereas false predictions of absence are dismissed as model errors (2).

Refinements of these biological models (3–5) are based on modifications of an equation describing transmission potential, expressed as the basic reproduction number  $R_0$ , which must equal at least 1 for disease persistence (13, 14). For an estimation of the correct value of  $R_0$  from which to predict malaria distribution, absolute, not relative, estimates of all quantities in the equation are needed. Instead, by omitting certain unquantified but important parameters and rearranging the equation (15), a relative measure of "epidemic potential" (EP) [now "transmission potential" (5)] has been derived as the reciprocal of the vector/host ratio required for disease persistence. This predicts a more extensive present-day distribution of malaria than is currently observed (12). The ratio of future EP to present EP is then presented as indicating the relative degree of the future risk of malaria, but this is an inappropriate measure of changing risk because a high ratio may still leave  $R_0 < 1$ .

Until such biological approaches can give accurate descriptions of the current situation of global malaria, they cannot be used to give reliable predictions about the future. Instead, an alternative two-step statistical approach to mapping vector-borne diseases gave a better description of the present global distribution of *falciparum* malaria and predicted remarkably few future changes, even under the most ex-

<sup>1</sup>Trypanosomiasis and Land-use in Africa Research Group, <sup>2</sup>Oxford Tick Research Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

\*To whom correspondence should be addressed. E-mail: david.rogers@zoology.ox.ac.uk

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# United States Patent [19]

Johnson

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[45] Sept. 6, 1977

[54] PRINTED REAGENT TEST DEVICES AND  
METHOD OF MAKING SAME

[75] Inventor: Leighton Clifford Johnson,  
Edwardsburg, Mich.

[73] Assignee: Miles Laboratories, Inc., Elkhart,  
Ind.

[21] Appl. No.: 701,403

[22] Filed: June 30, 1976

[51] Int. Cl.<sup>2</sup> ..... A61K 9/70; B05D 5/00;  
G01N 31/22; G01N 33/16

[52] U.S. Cl. .... 23/253 TP; 195/103.5 R;  
427/2; 427/288

[58] Field of Search ..... 23/253 TP; 73/356;  
116/114 AM; 195/103.5; 427/2, 288

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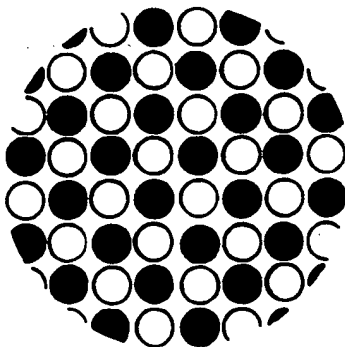
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Primary Examiner—Robert M. Reese  
Attorney, Agent, or Firm—E. H. Gorman, Jr.

## [57] ABSTRACT

A test device for determining the presence of a constituent in a sample, and a method for making it are disclosed. The test device comprises reactants (e.g. reagents, enzymes, etc.) incorporated with a carrier matrix such that when the device is wetted with a test sample, the reactants and the constituent react to produce a detectable response. The reactants are positioned separately from each other on the matrix in substantially, discrete, non-contacting areas. Hence, reactants are maintained substantially separate from each other until the test device is wetted with the sample.

12 Claims, 5 Drawing Figures



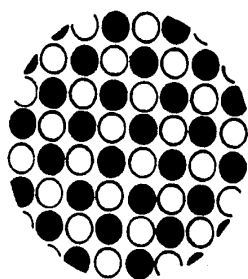


FIG. 1

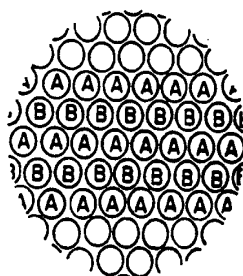


FIG. 2

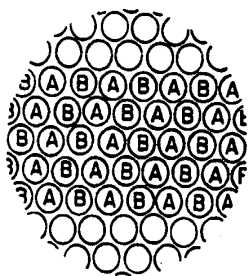


FIG. 3

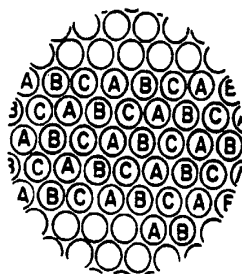


FIG. 4

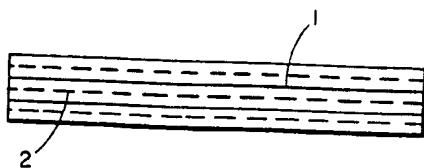


FIG. 5

# PRINTED REAGENT TEST DEVICES AND METHOD OF MAKING SAME

## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

The present invention relates to a test device for determining the presence of a constituent in a sample. The invention as defined by the claims comprises a test device, and a method for preparing it, in which potentially incompatible reactants are kept separate from each other until the actual testing of a sample, such as bodily fluid, takes place. Hence, the device comprises a carrier matrix incorporated with at least two reactants capable of interacting with a sample constituent being analyzed to produce a detectable response. The reactants are positioned separate from each other in substantially discrete, non-contacting areas on the carrier matrix.

### 2. Description of the Prior Art

The burgeoning field of test devices in the form of test strips has provided convenient and rapid analysis of various types of samples, including samples of biological, industrial, and automotive fluids, and the like. Diagnostic devices designed for detecting various clinically significant substances or constituents in biological fluids, such as urine and blood, including lysed or unlysed blood, blood plasma, and blood serum, have in many cases supplanted prior wet chemistry techniques which were both cumbersome and time-consuming. These diagnostic devices have thus assisted in the fast and accurate diagnosis and treatment of disease.

Conventional test strips generally comprise an absorbent or porous matrix incorporated with indicator reactants, usually of a colorimetric type. The sample to be tested is contacted with the matrix, such as by momentary immersion where the sample is liquid, and the indicator response is observed after a set period of time. For example, in a reagent strip for the detection of occult blood in urine a diagnostic strip can be employed which comprises an absorbent paper impregnated with o-tolidine and a peroxide. When this strip is wetted with urine containing occult blood, decomposition of the peroxide occurs with the accompanying oxidation of the o-tolidine to produce a color response. This test is sensitive and extremely useful in diagnosing urinary tract disorders. However, because of the relative incompatibility of employed reactants, shelf life has often been found to be relatively short and the strips can lose their sensitivity after long periods of storage.

Similar problems of reactant incompatibility occur in many other types of strips where more than one chemical reaction is involved. For example, reactants for testing ketone, blood urea nitrogen (BUN), and galactose levels have been known to have limited shelf lives. In order to explore ways of extending the shelf life of reagent test strips, i.e. methods of reducing the relative incompatibility of reactants, experiments were conducted to determine whether it would be possible to physically separate incompatible reagents on the strip itself. Prior to this work the successful separation of incompatible reagents had not been reported. The experiments were successful and it was found that reagent strips could indeed be prepared in which incompatible reactants were physically separated until becoming contacted with the sample to be analyzed. Strips prepared in accordance with the present invention have excellent shelf life and are vastly superior in this respect

to present commercial strips containing the same reactants.

## SUMMARY OF THE INVENTION

The present invention as defined by the claims relates to a test device for detecting a constituent in a sample, particularly a bodily fluid. The device comprises a carrier matrix incorporated with at least two different reactants capable of reacting with the constituent to produce a detectable response, the different reactants being physically separated from each other on the matrix. The different reactants are printed separately on the matrix as a plurality of substantially discrete areas, including dots, microdots, lines or the like.

## BRIEF DESCRIPTION OF THE DRAWINGS

Other and further advantages and features of the invention will be apparent to those skilled in the art from the following detailed description thereof, taken in conjunction with the accompanying drawings, in which:

FIGS. 1 through 5 are diagrammatic illustrations of different patterns for applying reactants in accordance with the present invention.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention at least two reactant materials are arranged separately in recurring patterns on a matrix. Circular dots represent an optimum shape from the standpoint of packing density and reactant diffusions during an assay. The dots can be printed so as not to touch each other and dissimilar dots can be present in equal or unequal numbers as desired. Printed lines of varying widths and other patterns such as special symbols can be substituted for dots if desired.

Referring to FIG. 1, one reactant is depicted as white dots, the second reactant being depicted as juxtaposed black dots. However, this arrangement is not the densest dot arrangement. Dots of the same reactant can be placed in a line (as A and B in FIG. 2) or can alternate on a line (as A and B in FIG. 3). FIG. 4 depicts a further pattern which is suitable where three different reactants (A, B and C) are employed. In FIG. 4 any reactant dot has six nearest neighbors which comprise three dots of each of the other two reactants.

Another useful technique in separating the printed reactants is the application of stripes to a carrier matrix. Hence, for a two reactant system, alternate stripes of the complimentary reactants can be laid out parallel on a matrix as in FIG. 5, in which solid lines 1 represent a first reactant and dotted lines 2 represent a second reactant.

The present invention has also been found to exhibit great utility in the deposition of enzymes entrapped in a polymer matrix. Hence, enzymes such as glucose oxidase can be printed in an ink containing acrylamide and a photopolymerization initiator such as potassium persulfate. Such an ink could be printed as dots, stripes, or other configurations and exposed after printing with a Number 2 photo flood lamp to effect polymerization.

In one embodiment of this particular entrapping technique, a stock solution of monomer compound is prepared by dissolving 40g of acrylamide in 100ml of 0.1M phosphate buffer having a pH of 7.4. A cross linking reactant is prepared by dissolving 2.3g of N,N-methylenediacrylamide in 100ml of 0.1M phosphate buffer (pH 7.4). Gels can then be prepared by mixing

these solutions in desired proportions together with a solution of an enzyme such as glucose oxidase. This gel can then be printed onto an inert matrix in accordance with the present invention and cured in situ.

Other applications of entrapped enzymes will be easily ascertainable by one skilled in the art by reference to U.S. Pat. Nos. 3,788,950, 3,793,445, 3,841,971, 3,859,169, and 3,935,071. These and other references in the prior art discuss in detail the physical entrapment of enzymes as well as enzymes covalently bound to a substrate and such techniques are readily applicable to the present invention.

In each of the above embodiments, whether dots or stripes, it can be seen that when the test device is wetted the separated reactants will at that time combine and interact with the constituent being analyzed. Typical test reactant compositions are set forth in U.S. Pat. Nos. 3,438,737; 3,095,277; 3,212,855; 3,164,534; 3,050,373; 2,981,606; 3,123,443; 3,252,762; 3,290,117; 3,092,463; 3,012,976; 3,122,420; 3,453,180; 3,585,001; 3,585,004; and 3,447,905 which are incorporated herein by reference.

The test device of the present invention is optimally prepared using printing techniques. For example, a polystyrene matrix can be printed with a first reactant ink and subsequently printed with a second reactant ink such that the first and second inks are imprinted in substantially noncontacting, coplanar impressions. Thus, in the case of an occult blood test device, a first ink containing o-tolidine is silk screened as a plurality of dots onto a polystyrene matrix. Subsequently, a second plurality of dots, juxtaposed with the first, is silk screened onto the matrix. The second plurality comprises a reagent ink containing a peroxide such as cumene hydroperoxide.

In another embodiment of the present invention, offset printing techniques are employed. An example of how this technique is employed in the present invention is the use of a rubber stamp containing raised dots. The rubber stamp is inked with a first reactant ink and applied to the matrix leaving a dot impression of the first reactant. The second reactant is stamped similarly onto the matrix except that the dots are juxtaposed with those of the first reactants.

Still another technique of preparing the present test device is that of applying ribbons or stripes of reactants separately onto the matrix. Thus each reactant ink is laid down alternately as parallel thin bands or concentric circles.

Normally, it is desirable to maintain the indicator composition, e.g. dye, for indicating a color change point as one of the separate discrete areas on the test device. For sample testing based on pH changes, it may also be desirable to maintain a buffer as a separate area on the test device until testing occurs.

It will be obvious to a person skilled in the art that many printing techniques can find applicability to the present invention. For example, it would be feasible to employ rotogravure printing techniques, silk screening, and offset printing. Of the forgoing methods, silk screening has to date been found to be preferred.

While silk is a preferred material for screening, screens made of other materials can also be employed, such as screens of woven polyester, polyamide or metal threads. Usually, art-known silk screens coated with standard photosensitive resist materials are employed. After photographic exposure of the screen (e.g. in the desired dot pattern) the exposed screen is washed, leaving the exposed pattern for printing. This technique, of

course, is not novel and is too well known in the printing art to require further discussion herein. Perforated sheets made of a material such as plastic or thin metal can also be employed. A perforated sheet can be used in the same manner as a screen by placing the sheet over the inert matrix, applying reactant to it and drawing a doctor blade across it to spread the reactant material and force it through the holes thereby printing the matrix. The screens or sheets must be loaded precisely with respect to the inert matrix and the formulation of the reactants must be such that the desired result is achieved without the reactants running together during or after application. Regardless of the method employed, the size of the discrete reactant areas applied in known proportions can be varied from very small areas, e.g. microdot size, to relatively large size. The alignment of the printing apparatus obviously becomes more critical as the reactant areas become smaller and closer together.

The printing techniques described herein can be employed with any of the conventional inert matrices used heretofore in diagnostic test devices, such as paper, plastic, and combinations thereof. The particular inert matrix chosen must be one which adequately reflects incident light since test devices are read by visually judging the intensity of reflected light from such devices. Optically transparent matrix materials such as Trycite® polystyrene film made by Dow Chemical Co. may be employed.

If desired, paper used as a matrix can be coated to improve its light scattering efficiency and the printability of the paper, i.e. adherence of reactants. The surface of the paper can be white in color to reflect as much visible light of all wavelengths as possible. Obviously, a mat finish is preferred over a high gloss finish.

A plastic matrix can have essentially the same optical characteristics as noted for paper. While plastic has the advantage of being less chemically reactive and more uniformly reflective, adherence of reactants can be somewhat more difficult on plastic than paper using the printing techniques described herein. If desired a white pigment can be incorporated with the plastic to achieve a desired reflective surface.

Known diluent substances useful to reduce hygroscopicity of reactants, such as chloroform, carbon tetrachloride, benzene, and the like; as well as known wetting agents, such as diglycol laurate, organic phosphate esters of anionic detergents in ethanol and the like, which aid in producing an even diffusion of color on a test device can be incorporated into the printed reactant compositions of this invention.

Test devices in accordance with this invention can advantageously be made in the form of long strips or tapes that are rolled up and inserted in a suitable roll-tape dispenser as well as being cut into individual test strips.

The following examples are presented in order to more clearly describe the invention and to point out preferred embodiments. They are not, however, intended in any way to limit the present invention and are not to be thus interpreted.

#### EXAMPLE I

##### Alternate Stripes

Two reactant inks were prepared as follows:

Polymer solution: A solution of cellulose acetate in acetone was prepared. This solution served separately as the vehicle for each reactant.

Reactant 1:

In 65 ml. of water was dissolved 2.8 g sodium citrate and 4.7 g. citric acid. Next was added 50 mg of Tetrabromophenol Blue and 30 ml. of methanol. The pH was then adjusted to 3.3 by the addition of a buffer. 10 ml. of the resulting mixture was added to 20 ml of the polymer solution to produce a first reactant ink.

Reactant 2:

2.2 g of sodium citrate and 10 mg of orthocresol sulfonephthaline in 6 ml ethanol were added to 24 ml of water and the pH was adjusted to 7.8 through the addition of a buffer. 10 ml of this mixture was added to 20 ml of the polymer solution to form a second reactant ink.

A small dispensing head was prepared for applying the two inks to a polystyrene matrix. The dispensing head was provided with 14 channels approximately 0.03 inch in width. Two dispensing ports fed alternate sets of channels. Hence, the first port provided one ink sample to odd channels 1,3,5 . . . 13, the second provided the other ink sample to even channels 2,4,6 . . . 14. A portion of each ink was added to its respective port and the dispensing head was drawn across a white polystyrene matrix, thus depositing alternating stripes of the first and second inks. The striped polystyrene matrix was then cut into strips about  $\frac{1}{4}$  inch in width. These were tested with a 100 mg percent albumin solution in water and in pure water. The strip which was dipped in the albumin solution yielded a yellow/green color whereas an identical strip dipped in water became pale yellow.

### EXAMPLE 2

#### Offset Dot Printing (Halftone)

This experiment was performed in order to demonstrate the feasibility of offset dot printing of reactants. A rubber plate for the printing was purchased from a local rubber stamping manufacturer. The manufacture of rubber plates is well known in the rubber stamp art, and the particular one purchased for this experiment was made using a metallic brass die containing normal printing periods as recessed dots. The periods were 30 mils in diameter and were squarely arranged in a density of 64 periods per square inch. An unvulcanized rubber matrix was placed over the die and pressure was applied causing the rubber to flow into the recesses of the metal die. Subsequently, heat was applied to the rubber to vulcanize it, causing the rubber plate to achieve a permanent configuration. The rubber sheet was then stripped off thus forming the rubber plate.

The rubber plate was cut into two squares,  $\frac{1}{4}$  inch on the side, thus forming the dies used for printing the reactant dots. Each rubber die was mounted using rubber cement to the face of an aluminum adapter used for mounting the die on a small arbor press which was commercially purchased.

Two reactant inks were prepared as in Example 1 for use with the dies. One of the dies was mounted on the press, and a piece of Trycite® polystyrene film obtained from Dow Chemical Co. was mounted in the press beneath the die. The die was inked with the first reactant ink and an impression of the ink was made on the polystyrene film. The second die was then inked with the second reactant ink and mounted in the press. The register of the plastic film was changed such that

the dots from the second die would be applied in a position juxtaposed with the first dots. The printing of the second dots completed the preparation of the test device, which was then air dried.

### EXAMPLE 3

#### Silk Screen Dot Printing

This experiment demonstrates the application of the present invention to the silk screening printing process. A standard silk screen was purchased from Dec-O-Art, Inc. in Elkhart, Indiana. This screen was photographically prepared by that corporation and contained patterns of 25 mil dots, 250 per square inch, and 40 mil dots, 125 per square inch. Thus, in the dot patterns on the silk screen, the dots constituted holes where an ink could flow through the screen, whereas all other areas in the dot pattern were closed to ink flow.

A sheet of Trycite polystyrene film obtained from Dow Chemical Co. was placed under the screen at a distance of from 1/16 to 3/16 inch. A portion of the first reactant ink from Example 1 was then drawn across the screen with the use of a square edged polyurethane squeegee. By exerting pressure on the squeegee, the screen contacted the polystyrene and a series of dots were laid down approximately the same size as the openings in the screen. The screen was then cleaned and a second series of dots was placed on the polystyrene using the second reactant ink of Example 1 except that the register of the screen was changed so that the second dots were juxtaposed with the first. The printed polystyrene sheet was then dried at ambient conditions, to form a usable printed test device.

The technique was repeated with the larger dots to likewise yield a satisfactory test device.

From the foregoing, it will be seen that this invention is well adapted to obtain all of the advantages hereinabove set forth, together with other advantages which are obvious and inherent to the system. The invention provides a rapid and relatively inexpensive method of applying reactants to a test device in a manner which prevents interaction of reactants until the test device is contacted with a sample to be tested. The shelf life of the test device is significantly improved.

Obviously, many other modifications and variations of the invention as hereinbefore set forth may be made without departing from the spirit and scope thereof.

What is claimed is:

1. In a test device for determining the presence of a constituent in a sample in which a carrier matrix is incorporated with a reagent system capable of interacting with the constituent to produce a detectable response, said system comprising at least two reactants, the improvement wherein at least two of the reactants are printed separately from each other in substantially discrete, non-contacting, substantially coplanar areas of the carrier matrix.

2. The improvement of claim 1 in which the constituent is occult blood, one reactant is o-tolidine and another separately printed reactant is a peroxide.

3. The improvement of claim 1 in which the reactants are present on the matrix as a plurality of substantially discrete dots.

4. The improvement of claim 3 in which the dots are positioned in alternate rows of different reactants.

5. The improvement of claim 1 in which the reactants are present on the matrix as a plurality of parallel stripes.

6. The improvement of claim 5 in which the stripes alternately contain a different reactant.

7. A test device for the detection of a constituent in a bodily fluid comprising a carrier matrix having a surface, and a reagent system comprising at least two reactant materials, said materials being separately printed in discrete areas on said surface in substantially discrete, non-contacting relation.

8. The test device of claim 7 in which one of the printed reactant materials is an enzyme entrapped in a polymer matrix.

9. The test device of claim 7 in which one of the reactant materials is an enzyme covalently bound to a substrate.

10. The test device of claim 7 in which the discrete relation comprises a recurring pattern.

11. The test device of claim 7 in which the discrete relation comprises dots, and the carrier matrix is paper.

12. The test device of claim 7 in which the discrete relation comprises dots, and the carrier matrix is plastic.

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**Brennan**

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[45] **Date of Patent:** **Nov. 16, 1999**

[54] **METHOD AND APPARATUS FOR  
 CONDUCTING AN ARRAY OF CHEMICAL  
 REACTIONS ON A SUPPORT SURFACE**

90/03382 4/1990 WIPO .  
 WO 90/03382 4/1990 WIPO .  
 WO 90/15070 12/1990 WIPO .

[75] **Inventor:** **Thomas M. Brennan, San Francisco,  
 Calif.**

[73] **Assignee:** **Proteogene Laboratories, Inc., Palo  
 Alto, Calif.**

[21] **Appl. No.:** **08/465,761**

[22] **Filed:** **Jun. 6, 1995**

#### Related U.S. Application Data

[63] Continuation of application No. 08/068,540, May 27, 1993,  
 Pat. No. 5,474,796, which is a continuation-in-part of appli-  
 cation No. 07/754,614, Sep. 4, 1991, abandoned.

[51] **Int. Cl.<sup>6</sup>** ..... **C12Q 1/68**

[52] **U.S. Cl.** ..... **435/6; 435/91.2**

[58] **Field of Search** ..... **427/2.13, 2.11,  
 427/264, 266, 271, 282, 336, 338, 407.2;  
 435/285.1, 317.1, 283.1, 91.2, 6; 422/57,  
 58, 99, 104**

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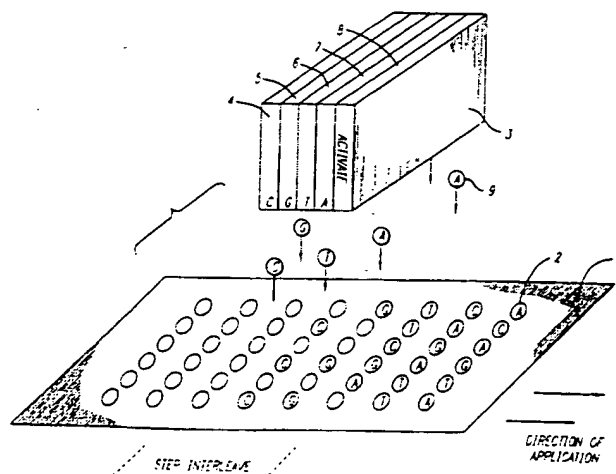
*Primary Examiner*—Scott W. Houtteman

*Attorney, Agent, or Firm*—Albert P. Halluin; John A.  
 Bendrick; Howrey & Simon

[57] **ABSTRACT**

The invention provides apparatus and methods for making  
 arrays of functionalized binding sites on a support surface.  
 The invention further provides apparatus and methods for  
 sequencing oligonucleotides and for identifying the amino  
 acid sequence of peptides that bind to biologically active  
 macromolecules, by specifically binding biologically active  
 macromolecules to arrays of peptides or peptide mimetics.

**15 Claims, 8 Drawing Sheets**



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A C

Matrix

(a)

G T

AAA	AAC	ACA	ACC	CAA	CAC	CCA	CCC
AAG	AAT	ACG	ACT	CAG	CAT	CCG	CCT
AGA	AGC	ATA	ATC	CGA	CGC	CTA	CTC
AGG	AGT	ATG	<u>ATT</u>	CGG	CGT	CTG	<u>CTT</u>
GAA	GAC	GCA	<u>GCC</u>	TAA	TAC	TCA	<u>TCC</u>
GAG	GAT	GCG	GCT	TAG	TAT	TCG	<u>TCT</u>
GGA	GGC	GTA	GTC	TGA	TGC	<u>TTA</u>	<u>TTC</u>
GGG	GGT	GTG	<u>GTT</u>	TGG	<u>TGT</u>	<u>TTG</u>	<u>TTT</u>

(b)

DNA Fragment

-----ATTCTTGTTA---

ATT

TTC

TTA, TTG

TCT

CTT

TTG

TTA

(c)

TGT

GTT

TTA

Correct Assembly

Possible N+1 List

FIG. 1

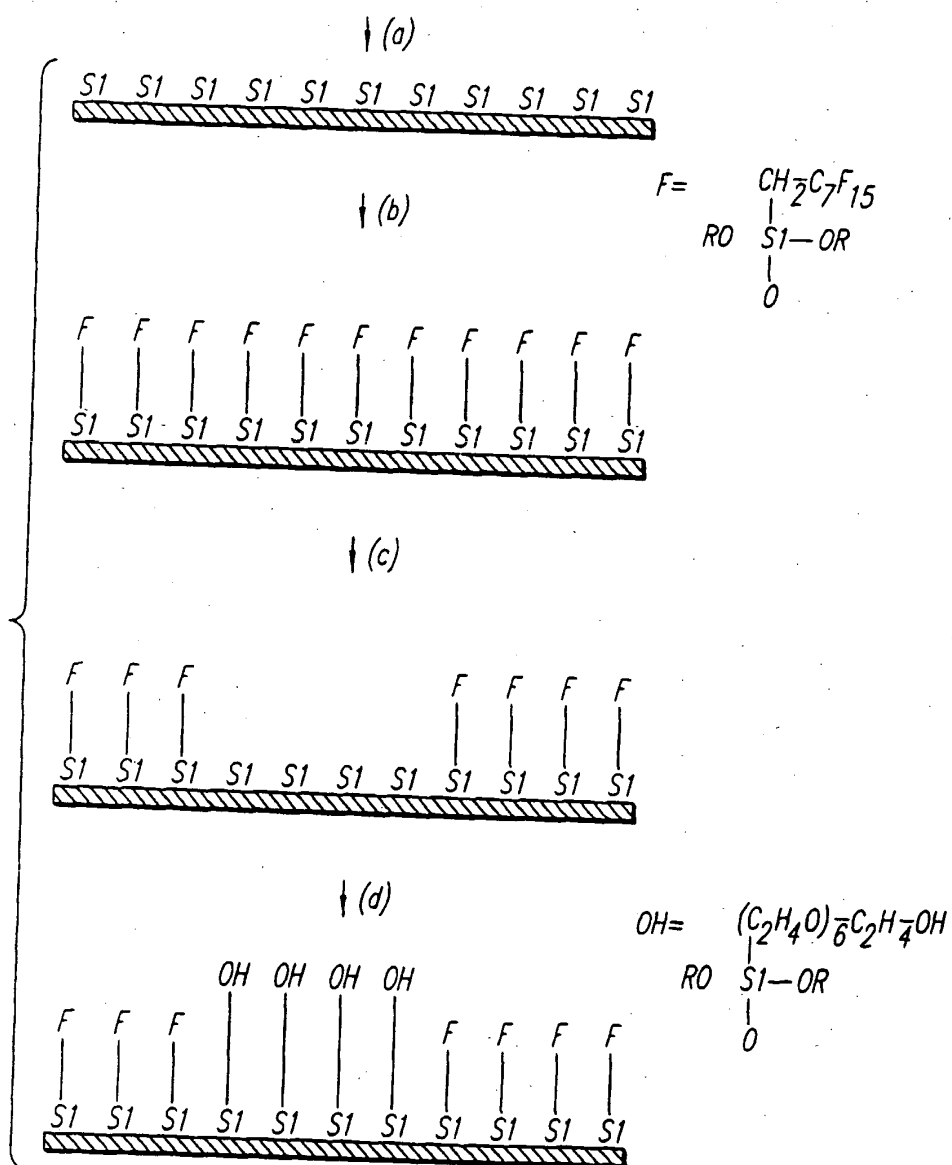
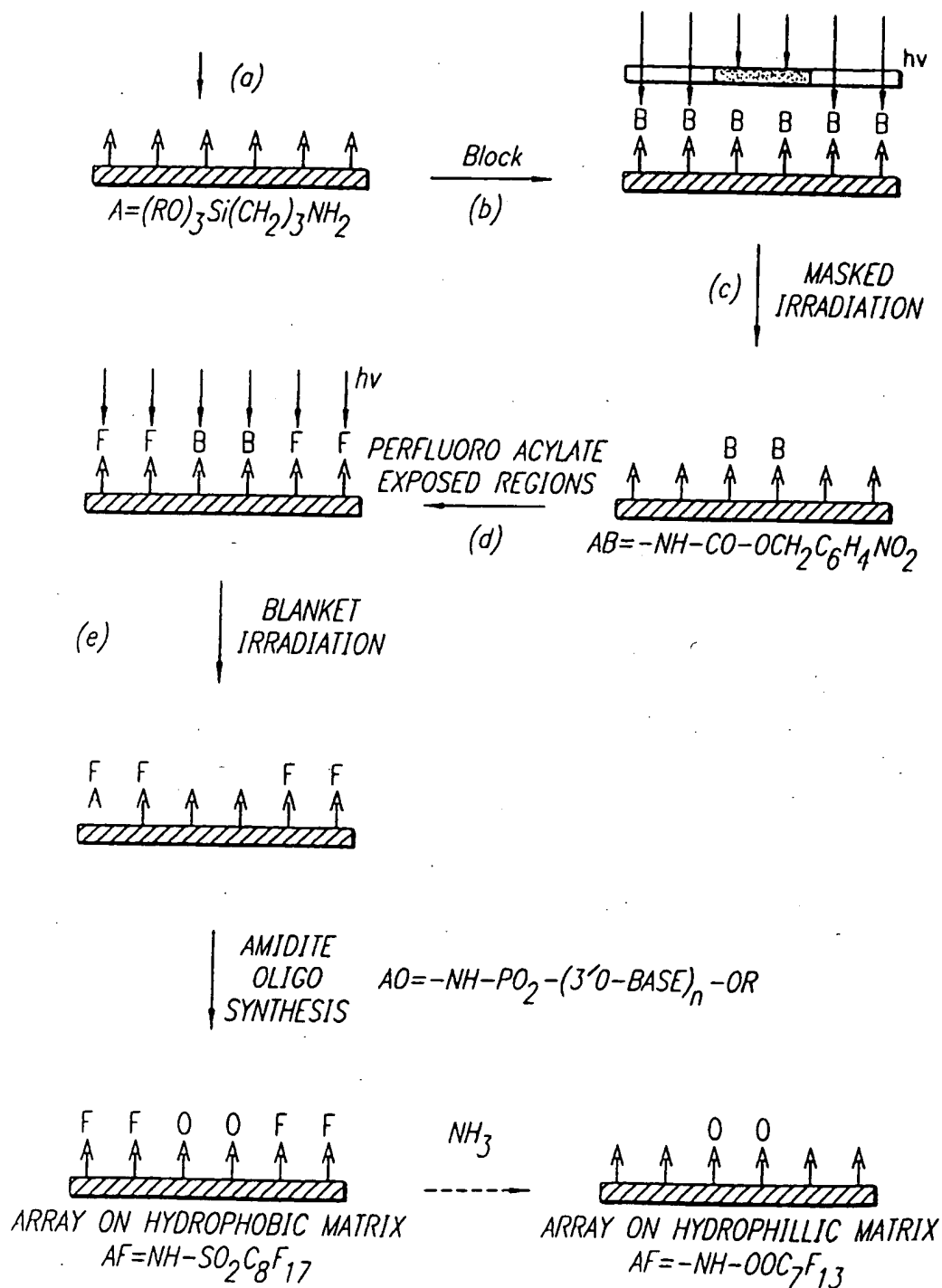


FIG. 2A

FIG. 2B



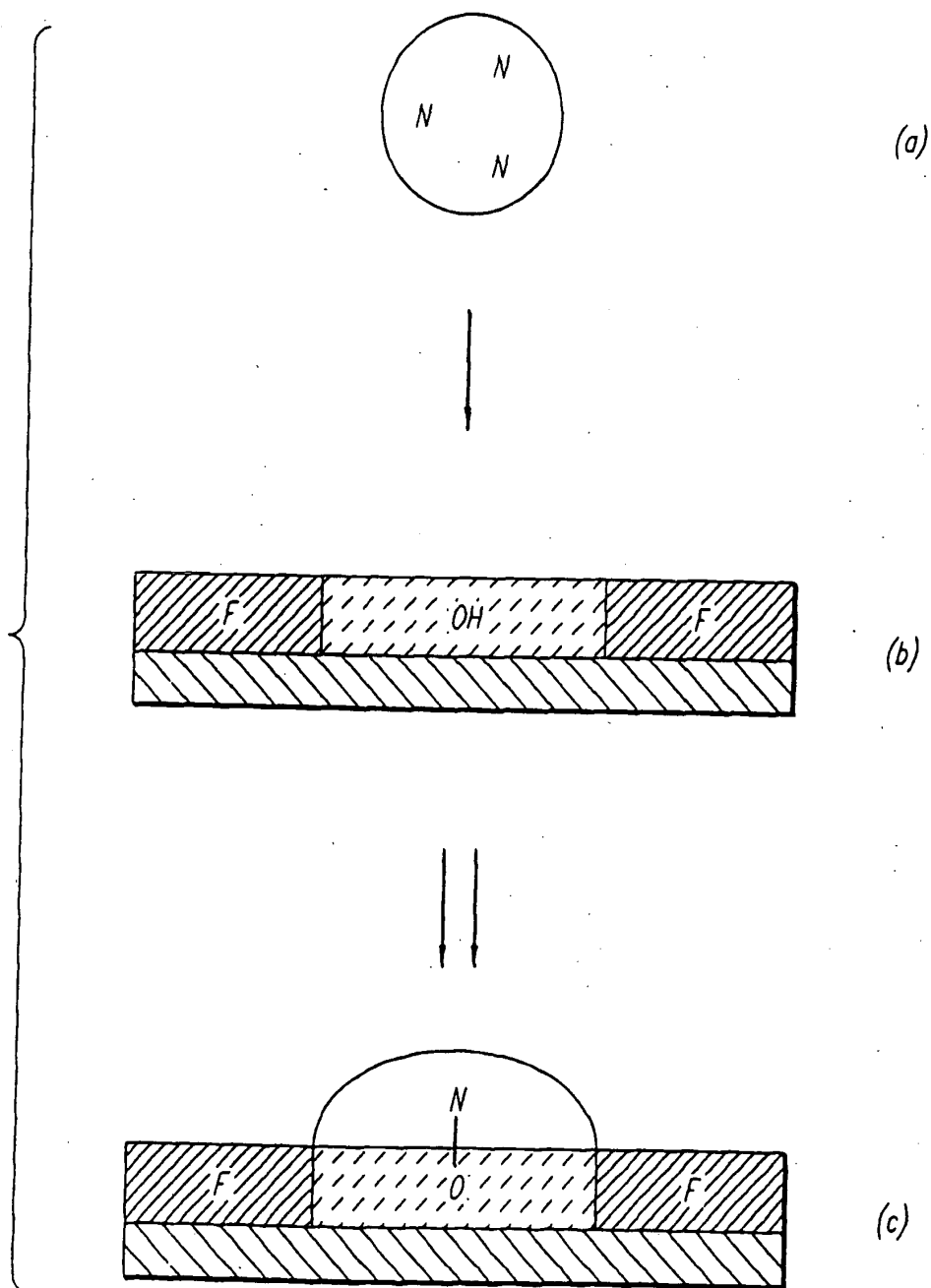


FIG. 3

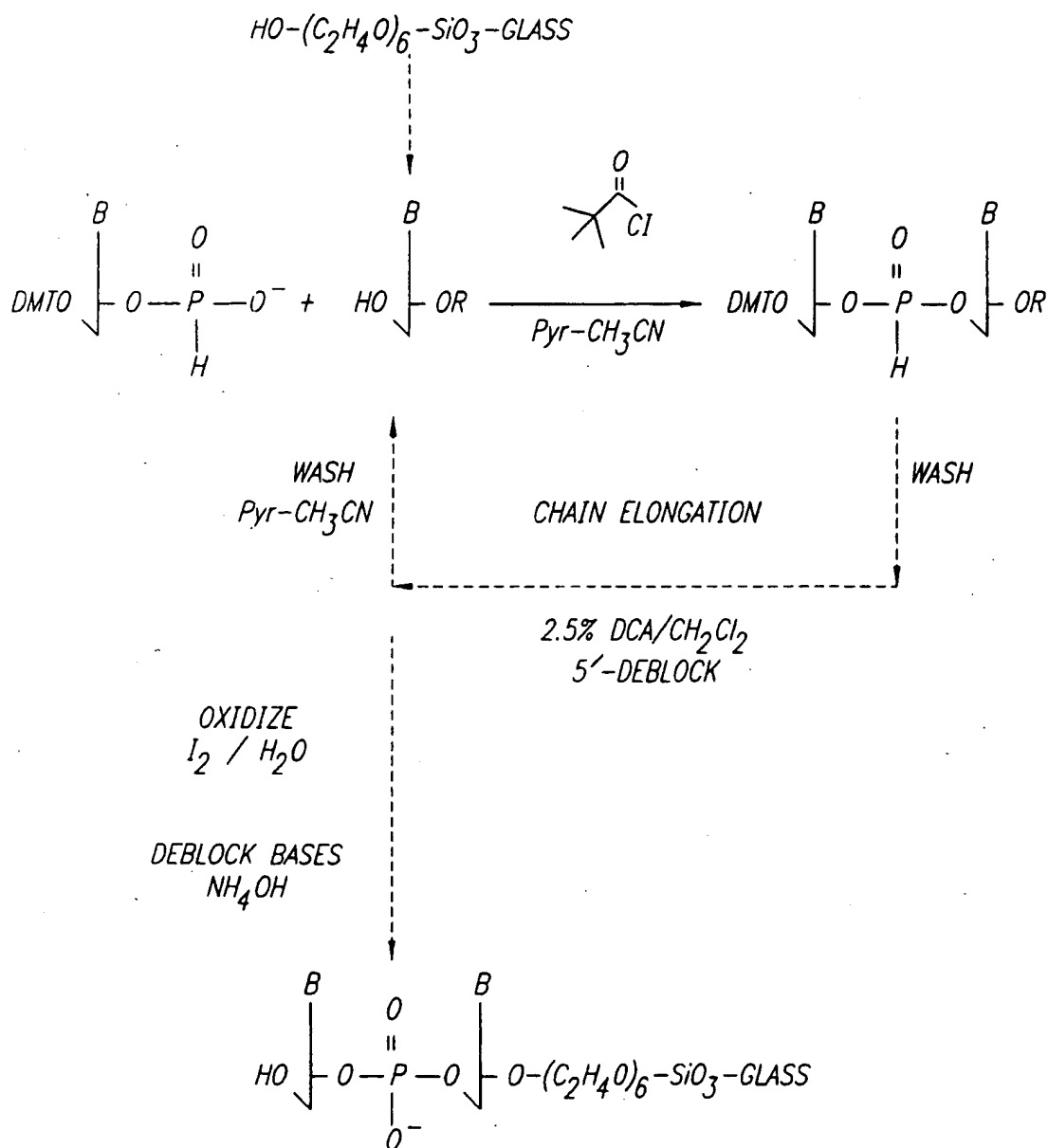


FIG. 4

FIG. 5A

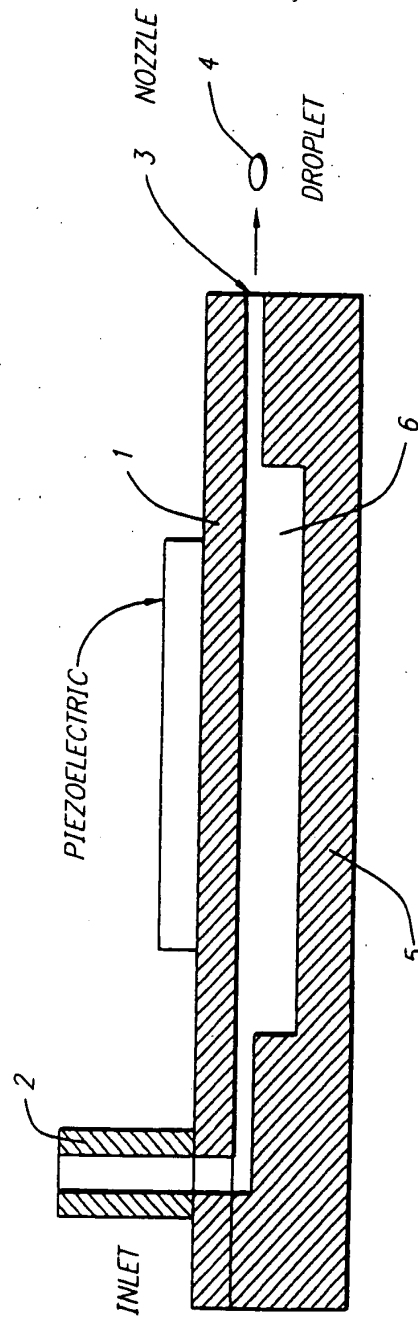
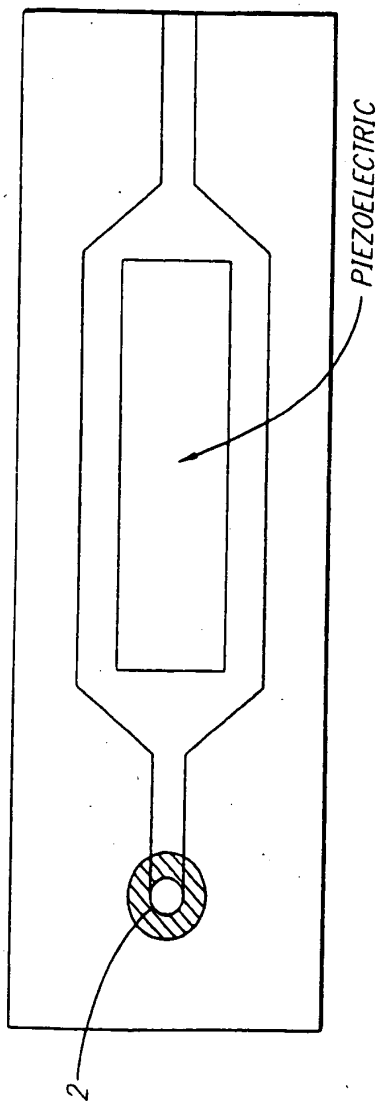


FIG. 5B



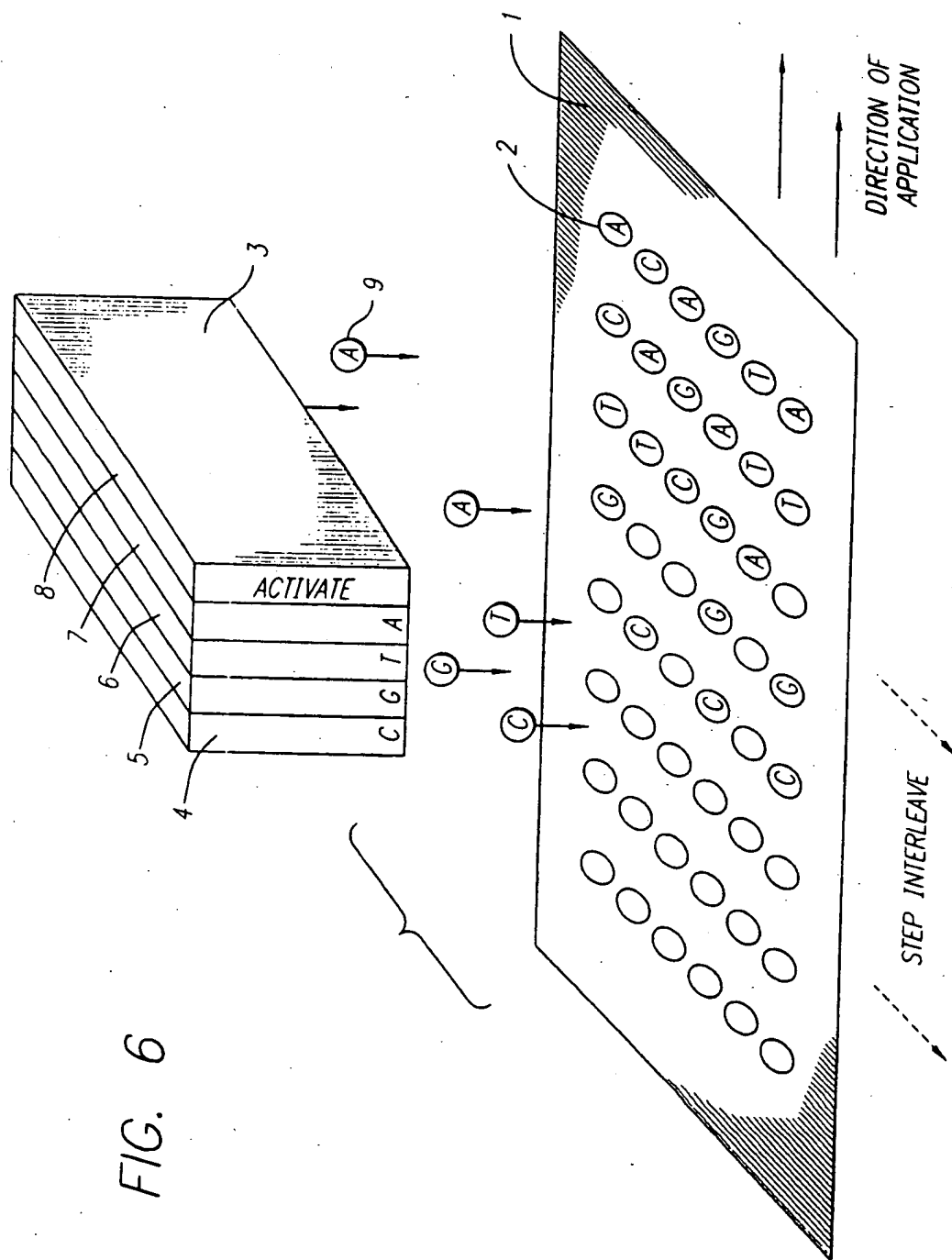
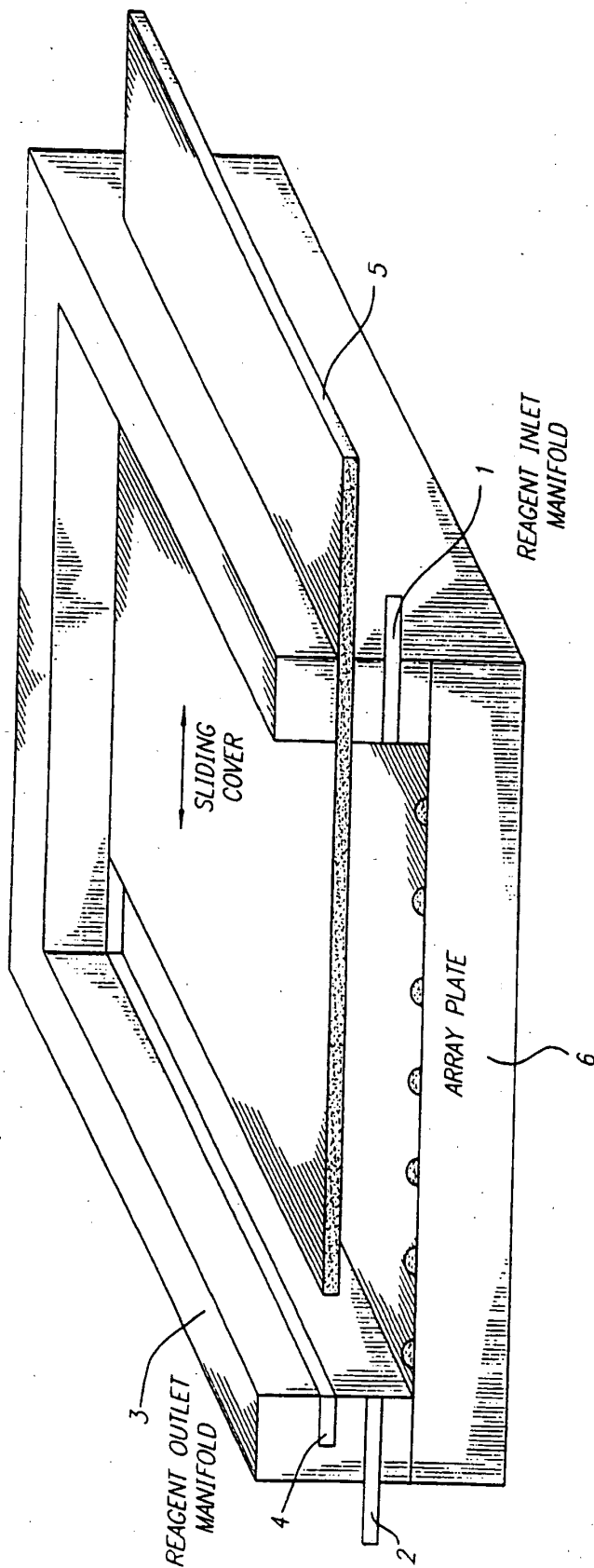


FIG. 7



# 1 **METHOD AND APPARATUS FOR CONDUCTING AN ARRAY OF CHEMICAL REACTIONS ON A SUPPORT SURFACE**

This is a continuation of application Ser. No. 08/068,540, filed May 27, 1993; now U.S. Pat. No. 5,474,796 which in turn is a continuation-in-part of application Ser. No. 07/754,614, filed Sep. 4, 1991 now abandoned.

## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

The invention relates to methods for conducting a large number of chemical reactions on a support surface, methods for making the support surface, and the support surface itself.

### 2. Summary of the Related Art

Proposals for the direct sequencing of DNA by hybridization with arrays of oligonucleotides are known in the art. Drmanac et al., *Genomics* 4; 114 (1989) proposes hybridization array-mediated DNA sequencing by binding target DNA to a dot blot membrane, followed by probing with an array of oligonucleotides. Khrapko et al., *FEBS Letters* 256, 118 (1989) proposes hybridization array-mediated DNA sequencing by binding the oligonucleotide array to a support membrane, followed by probing with target DNA.

Synthesis of arrays of bound oligonucleotides or peptides is also known in the art. Houghton, in the Multiple Peptide System product brochure describes the T-bag method, in which an array of beads is physically sorted after each interaction. This method becomes unwieldy for the preparation of large arrays of oligonucleotides. Geysen et al., *J. Immunol. Methods* 102; 259 (1987) discloses the pin method for the preparation of peptide arrays. The density of arrays that may be produced by this method is limited, and the dipping procedure employed in the method is cumbersome in practice. Southern, *Genome Mapping and Sequencing Conference*, May 1991, Cold Spring Harbor, N.Y., disclosed a scheme for oligonucleotide array synthesis in which selected areas on a glass plate are physically masked and the desired chemical reaction is carried out on the unmasked portion of the plate. In this method it is necessary to remove old mask and apply a new one after each interaction. Fodor et al., *Science* 251; 767 (1991) describes a method for synthesizing very dense 50 micron arrays of peptides (and potentially oligonucleotides) using mask-directed photochemical deprotection of synthetic intermediates. This method is limited by the slow rate of photochemical deprotection and by the susceptibility to side reactions (e.g., thymidine dimer formation) in oligonucleotide synthesis. Khrapko et al., *FEBS Letters* 256; 118 (1989) suggests simplified synthesis and immobilization of multiple oligonucleotides by direct synthesis on a two dimensional support, using a printer-like device capable of sampling each of the four nucleotides into given dots on the matrix. However, no particulars about how to make or use such a device are provided.

Some methods for permanently attaching oligonucleotides to glass plates in a manner suitable for oligonucleotide synthesis are known in the art. Souther, *Chem. abst.* 113; 152979r (1990) describes a stable phosphate ester linkage for permanent attachment of oligonucleotides to a glass surface. Mandenius et al., *Anal. Biochem.* 157; 283 (1986) teaches that the hydroxyalkyl group resembles the 5'-hydroxyl of oligonucleotides and provides a stable anchor on which to initiate solid phase synthesis.

The related art contains numerous ideas and information related to arrays of chemical reactants on a solid support.

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However, existing or suggested methods are limited, and do not conveniently and reliably produce the very large, high density arrays. There is, therefore, a need for new methods for preparing large high density arrays of reactive sites. Ideally, such methods should utilize relatively simple machinery to produce large, dense arrays of solid phase bound reactants in a reproducible and rapid manner.

## SUMMARY OF THE INVENTION

This invention provides a method for conducting a large number of chemical reactions on a support surface. Solutions of chemical reactants are added to functionalized binding sites on the support surface by means of a piezoelectric pump. This pump deposits microdroplets of chemical reactant solution onto the binding sites. The chemical reactant at each binding site is separated from the others by surface tension. Typically, the support surface has  $10^4$  functionalized binding sites per  $\text{cm}^2$  and each functionalized binding site is about 50–2000 microns in diameter. Typically, the amounts of reagents added to each binding site is in a volume of about 50 picoliter to 2 microliter. The reactions at the functionalized binding site may form covalent bonds such as esters or amide bonds or may involve non-covalent specific binding reactions such as antibody/antigen binding or oligonucleotide specific binding. The invention also includes array plates and methods for making the array plates.

Typically, the array plates are made by the process set out in FIG. 2A by

- (a) coating a support surface with a positive or negative photoresist substance which is subsequently exposed and developed to create a patterned region of a first exposed support surface;
- (b) reacting the first support surface with a fluoroalkylsilane to form a stable fluoroalkylsiloxane hydrophobic matrix on the first support surface;
- (c) removing the remaining photoresist to expose a second support surface; and
- (d) reacting the second support with a hydroxy or aminoalkylsilane to form derivatized hydrophilic binding site regions.

The preferred siloxane reaction product of the present invention is tridecafluoro-1,1,2,2-tetrahydrooctyl siloxane. In FIG. 2A, the hatched lines are the solid support, "S1" represents a first exposed support surface site, "S1-F" is a hydrophobic fluoroalkylsilane site, and "S1-OH" is a derivatized hydrophilic binding site.

Alternatively, the array plates can be made by the process set out in FIG. 2B by

- (a) reacting a support surface with a hydroxy or aminoalkylsilane to form a derivatized hydrophilic support surface;
- (b) reacting the support surface from step (a) with *o*-nitrobenzyl carbonyl chloride as a temporary photolabile blocking to provide a photoblocked support surface;
- (c) exposing the photoblocked support surface of step (b) to light through a mask to create unblocked areas on the support surface with unblocked hydroxy or aminoalkylsilane;
- (d) reacting the exposed surface of step (c) with perfluoroalkanyl halide or perfluoroalkylsulfonyl halide to form a stable hydrophobic (perfluoroacyl or perfluoroalkylsulfonamido) alkyl siloxane matrix; and
- (e) exposing this remaining photoblocked support surface to create patterned regions of the unblocked hydroxy-

or aminoalkylsilane to form the derivatized hydrophilic binding site regions.

The preferred siloxanes of the present invention are 3-perfluorooctanoyloxy propylsiloxane and 3-perfluorooctanesulfonamido propylsiloxane. In FIG. 2B, the hatched lines are the solid support, "A" represents a hydrophilic support site, "A B" represents a temporary photolabile blocked support site, and "A F" represents a hydrophobic site.

The invention also provides a method for determining or confirming the nucleotide sequence of a target nucleic acid. The target nucleic acid is labelled by conventional methods and hybridized to an oligonucleotides of known sequence previously bound to sites on the array plate. The array plate having bound labelled target nucleic acid is then washed at appropriate stringency and the presence and location of bound labelled target nucleic acid is determined using scanning analyzers. Since the sequence of the covalently attached oligonucleotide in each element on the array is known, this allows the unambiguous determination of the nucleotide sequence of the target nucleic acid.

The methods of the invention may also be applied to the determination of peptides or peptide mimetics that bind biologically active receptors. In this aspect, peptide arrays of known sequence can be applied to glass plates using the same piezoelectric pump/surface tension wall method described supra. The resulting array of peptides can then be used in binding analyses with biologically active receptor ligands to screen for peptide mimetics of receptor agonists and antagonists. Thus, the invention provides a method for producing peptide array plates, peptide array plates having covalently bound peptides separated by surface tension areas, and methods of using such peptide array plates to screen for peptide mimetics of receptor agonists and antagonists.

Those skilled in this art will recognize a wide variety of binding site and chemical reactants for forming either covalent bonds or for specific binding reagents.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Hybridization analysis using arrays of trimers. Individual dots that have bound the DNA fragment are underlined.

FIG. 2A: Illustrates the formation of an array surface that is ready for solid phase synthesis.

FIG. 2B: Illustrates O-Nitrocarbamate array making chemistry.

FIG. 3: Surface tension wall effect at the dot-interstice interface. The droplet containing solid phase synthesis reagents does not spread beyond the perimeter of the dot due to the surface tension wall.

FIG. 4: Hydrogen-phosphonate solid phase oligonucleotide synthesis on an array surface prepared according to Example 1.

FIG. 5: Top and side views of a piezoelectric impulse jet of the type used to deliver solid phase synthesis reagents to individual dots in the array plate synthesis methods according to the invention.

FIG. 6: Use of a piezoelectric impulse jet head to deliver blocked nucleotides and activating agents to individual dots on an array plate. The configuration shown has a stationary head/moving plate assembly.

FIG. 7: Enclosure for array reactions showing array plate, sliding cover and manifolds for reagent inlet and outlet.

#### DETAILED DESCRIPTION OF THE INVENTION

The practice of present invention can include a number of photoresist substances. These substances are readily known

to those of skill in the art. For example, an optical positive photoresist substance (e.g., AZ 1350 (Novolac™ type-Hoechst Celanese™) (Novolac™ is a proprietary novolak resin, which is the reaction product of phenols with formaldehyde in an acid condensation medium)) or an E-beam positive photoresist substance (e.g., EB-9 (polymethacrylate by Hoya™)) can be used.

A number of siloxane functionalizing reagents can be used, for example:

1. Hydroxyalkyl siloxanes  
(Silylate surface, functionalize with diborane, and H<sub>2</sub>O<sub>2</sub> to oxidize the alcohol)
  - a. allyl trichlorochlorosilane → 3-hydroxypropyl
  - b. 7-oct-1-enyl trichlorochlorosilane → 8-hydroxyoctyl
2. Diol (dihydroxyalkyl) siloxanes  
(silylate surface, and hydrolyze to diol)
  - a. glycidyl trimethoxysilane → (2,3-dihydroxypropyloxy)propyl
3. Aminoalkyl siloxanes (amines require no intermediate functionalizing step)
  - a. 3-aminopropyl trimethoxysilane → 3-aminopropyl
4. Dimeric secondary aminoalkyl siloxanes
  - a. bis (3-trimethoxysilylpropyl) amine → bis (silyloxypropyl) amine

In addition, a number of alternative functionalized surfaces can be used in the present invention. These include the following:

1. Polyethylene/polypropylene functionalized by gamma irradiation or chromic acid oxidation, and reduction to hydroxyalkyl surface.
2. Highly crosslinked polystyrene-divinylbenzene derivatized by chloromethylation, and aminated to benzylamine functional surface.
3. Nylon—the terminal aminohexyl groups are directly reactive.
4. Etched, reduced polytetrafluoroethylene.

There are two important characteristics of the masked surfaces in patterned oligonucleotide synthesis. First, the masked surface must be inert to the conditions of ordinary oligonucleotide synthesis; the solid surface must present no free hydroxy, amino or carboxyl groups to the bulk solvent interface. Second, the surface must be poorly wet by common organic solvents such as acetonitrile and the glycol ethers, relative to the more polar functionalized binding sites.

The wetting phenomenon is a measure of the surface tension or attractive forces between molecules at a solid-liquid interface, and is defined in dynes/cm<sup>2</sup>. Fluorocarbons have very low surface tension because of the unique polarity (electronegativity) of the carbon-fluorine bond. In tightly structured Langmuir-Blodgett type films, surface tension of a layer is primarily determined by the percent of fluorine in the terminus of the alkyl chains. For tightly ordered films, a single terminal trifluoromethyl group will render a surface nearly as lipophobic as a perfluoroalkyl layer. When fluorocarbons are covalently attached to an underlying derivatized solid (highly crosslinked polymeric) support, the density of reactive sites will generally be lower than Langmuir-Blodgett and group density. However, the use of perfluoroalkyl masking agents preserves a relatively high fluorine content in the solvent accessible region of the supporting surface.

There are also two important characteristics of the derivatized regions in patterned oligonucleotide synthesis. The surface must be compatible with the method of detection of hybridization. Radioactivity is largely being replaced by spectroscopic, chemiluminescent and fluorescent detection

techniques in DNA research. It is desirable that the surface be optically transparent. A second important characteristic is that the linkage of the penultimate oligonucleotide to the surface have high chemical stability, at least equal to that of the polyphosphate backbone in DNA.

The optical properties of glass (polytetrasiloxane) are unsurpassed for detection purposes. Further, there are numerous techniques developed by the semiconductor industry using thick films (1-5 microns) of photoresists to generate masked patterns of exposed glass surfaces. The best method to derivatize the first exposed glass surface is with volatile fluoroalkyl silanes using gas phase diffusion to create closely packed lipophobic monolayers. The polymerized photoresist provides an effectively impermeable barrier to the gaseous fluoroalkyl silane during the time period of derivatization of the exposed region. Following lipophobic derivatization however, the remaining photoresist can be readily removed by dissolution in warm organic solvents (methyl, isobutyl, ketone, or N-methyl pyrrolidone) to expose a second surface of raw glass, while leaving the first applied silane layer intact. This second region glass can then be derivatized by either solution or gas phase methods with a second, polar silane which contains either a hydroxyl or amino group suitable for anchoring solid phase oligonucleotide synthesis.

Siloxanes have somewhat limited stability under strongly alkaline conditions. Conditions such as 0.1 N sodium hydroxide, typically employed to strip probes from nylon hybridization membranes, should be avoided for reusable glass based hybridization arrays.

Teflon (polytetrafluoroethylene) itself would provide an ideal lipophobic surface. Patterned derivatization of this type of material can be accomplished by reactive ion or plasma etching through a physical mask or using an electron beam, followed by reduction to surface hydroxymethyl groups. However, the opacity of teflon at visible wavelengths severely restrict the applicable methods for detection of hybridization.

Depending on the ultimate application, other organic polymers have desirable characteristics for patterned oligonucleotide synthesis. Polypropylene is relatively transparent to visible light. It can be surface derivatized by chromic acid oxidation, and converted to hydroxy- or aminomethylated surfaces which provide oligonucleotide synthesis anchors of high chemical stability. Highly crosslinked polystyrene-divinylbenzene (ca. 50%) is non-swellable, and can be readily surface derivatized by chloromethylation and subsequent functional group manipulation. Nylon provides an initial surface of hexylamino groups.

The lipophobic patterning of these surfaces can be effected using the same type of solution based thin film masking techniques and gas phase derivatization as glass, or by direct photochemical patterning using *o*-nitrobenzylcarbonyl blocking groups. Perfluoroalkyl carboxylic and sulfonic acid derivatives rather than silanes are now used to provide the lipophobic mask of the underlying surface during oligonucleotide synthesis.

The solution of chemical reactant can be added to the functionalized binding site through utilization of a piezoelectric pump (FIG. 5) in an amount where the solution of chemical reactant at each binding site is separate from the solution of chemical reactant at other binding sites by surface tension. As described more fully infra, in the pump depicted in FIG. 5, reactant solution is inserted through the inlet (2) into the chamber (6) formed between the upper (1) and lower (5) plates of the piezo. Application of a voltage difference across the upper and lower plates causes compression of the piezo, forcing a microdroplet (4) out through the nozzle (3).

FIG. 3 depicts the deposition of the reactant solution on a functionalized binding site and subsequent reaction with the surface. A micro-droplet of solution (FIG. 3(a)) is deposited on the functionalized binding site (center cross-hatched region in FIG. 3(b)). Because of the differences in wetting properties of the reactant solution on the functionalized binding site and the surrounding surface, the micro-droplet of the reactant solution beads on the functionalized binding site and the reactants in solution react with the surface (FIG. 3(c)).

The piezoelectric pump that may be utilized in the invention delivers minute droplets of liquid to a surface in a very precise manner. The pump design is similar to the pumps used in ink jet printing. The piezopump is capable of producing 50 micron or 65 picoliter droplets at up to 3000 Hz and can accurately hit a 250 micron target in a 900° C. oven at a distance of 2 cm in a draft free environment. Preferred embodiments of the apparatus according to the invention are set forth in Example 3.

Alternative pump designs should take into account the following physical and mechanical considerations for reliable performance to be obtained. When a non-compressible fluid inside of a pumping cavity is subjected to a rapid strong pressure pulse, the direction of flow of the liquid from the cavity is determined primarily by the inertial resistance of the liquid placed. There is more liquid, and thus resistance to flow, on the inlet side than through the nozzle port. The column of liquid that is forced out of the nozzle begins to neck off as a result of surface tension. The stream breaks as the piezoelectric is de-energized, with the remaining column of liquid drawn back into the nozzle. The droplet that has necked off continues its flight with the velocity it achieved in the initial acceleration. Typically, the ejection velocity is about 1-2 meters/sec.

In normal printing applications using 150 micron drops of viscous water-based inks, the head speed is typically about 0.5 meter/sec. This motion adds a transverse velocity component to the droplet trajectory and can affect aiming accuracy. It may also cause the drop to skip when it hits a surface. Droplets fired from a stationary head tend to evaporate more slowly because they follow in the vapor trail of the preceding drop. The heads work most reliably when the inlet supply lines are not required to flex and the liquids are not subjected to acceleration forces.

The size of the drop is determined primarily by the surface tension of the solution and by the diameter of the pump nozzle. The smaller the droplet, the faster it will evaporate and the more its trajectory will be affected by drafts. Nozzles smaller than 25 microns tend to become plugged with dust particles. For water, the drop diameter is approximately 1.5 times the nozzle diameter. Typically, drops will not vary in size by more than 5%. We have shown that the jet will also successfully eject a variety of polar solvents, including CH<sub>3</sub>CN and MeOH. With these less viscous solvents, too forceful an ejection pulse may result in the formation of a series of trailing satellite droplets in addition to the primary drop. The duration of the pulse also affect satelliting.

After the cavity has returned to its original state, a period of time must be allowed for the nozzle to refill by capillary action before another cycle of pulsing can be initiated. It is important for the nozzle refill only to the top of the orifice, but the liquid meniscus not spread out onto the front face of the jet. This is prevented by silanizing the face to reduce its surface tension. The head is also operated under slight negative pressure to prevent overfilling. The aim of the drop is in the axial direction of the nozzle, but defects in the face coating can affect the trajectory.

Arrays of nozzles with up to 64 independent pumping chambers but a common inlet supply have been fabricated. It is important that each chamber inlet have some restriction so that operation of one pumping chamber does not affect the others. The separation between nozzles is typically 400 microns for printing applications, but denser arrays can be produced either by interleaving the transverse motion of the target or decreasing the nozzle spacing.

#### EXAMPLE 1

##### Preparation of Array Plates Ready for Oligonucleotide or Peptide Assembly

The hybridization array is synthesized on a glass plate. The plate is first coated with the stable fluorosiloxane 3-(1,1-dihydroperfluorooctyloxy) propyltriethoxysilane. A CO<sub>2</sub> laser is used to ablate off regions of the fluorosiloxane and expose the underlying silicon dioxide glass. The plate is then coated with glycidyloxypropyl trimethoxysilane, which reacts only on the exposed regions of the glass to form a glycidyl epoxide. The plate is next treated with hexaethyleneglycol and sulfuric acid to convert the glycidyl epoxide into a hydroxyalkyl group, which acts as a linker arm. The hydroxyalkyl group resembles the 5'-hydroxide of nucleotides and provides a stable anchor on which to initiate solid phase synthesis. The hydroxyalkyl linker arm provides an average distance of 3-4 nm between the oligonucleotide and the glass surface. The siloxane linkage to the glass is completely stable to all acidic and basic deblocking conditions typically used in oligonucleotide or peptide synthesis. This scheme for preparing array plates is illustrated in FIGS. 2(A) and 2(B) and was previously discussed.

#### EXAMPLE 2

##### Assembly of Oligonucleotides on the Array Plates

The hydroxyalkylsiloxane surface in the dots has a surface tension of approximately  $\gamma=47$ , whereas the fluorosilane has a surface tension of  $\gamma=18$ . For oligonucleotide assembly, the solvents of choice are acetonitrile, which has a surface tension of  $\gamma=29$ , and diethylglycol dimethyl ether. The hydroxyalkylsiloxane surface is thus completely wet by acetonitrile, while the fluorosiloxane masked surface between the dots is very poorly wet by acetonitrile. Droplets of oligonucleotide synthesis reagents in acetonitrile are applied to the dot surfaces and tend to bead up, as shown in FIG. 3. Mixing between adjacent dots is prevented by the very hydrophobic barrier of the mask. The contact angle for acetonitrile at the mask-dot interface is approximately  $\theta=43^\circ$ . The plate effectively acts as an array microliter dish, wherein the individual wells are defined by surface tension rather than gravity. The volume of a 40 micron droplet is 33 picoliter. The maximum volume retained by a 50 micron dot is approximately 100 picoliter, or about 3 droplets. A 100 micron dot retains approximately 400 picoliter, or about 12 droplets. At maximum loading, 50 micron and 100 micron dots bind about 0.07 and 0.27 femtomoles oligonucleotide, respectively.

Assembly of oligonucleotides on the prepared dots (FIG. 2B, bottom) is carried out according to the H-phosphonate procedure (FIG. 4), or by the phosphoramidite method. Both methods are well known to those of ordinary skill in the art. Oligonucleotide and Analogs, A Practical Approach (F. Eckstein ed., 1991). Delivery of the appropriate blocked nucleotides and activating agents in acetonitrile is directed to individual dots using the picopump apparatus described in

Example 3. All other steps, (e.g., DMT deblocking, washing) are performed on the array in a batch process by flooding the surface with the appropriate reagents. An eight nozzle piezoelectric pump head is used to deliver the blocked nucleotides and activating reagents to the individual dots, and delivering droplets at 1000 Hz, requires only 32 seconds to lay down a 512x512 (262 k) array. Since none of the coupling steps have critical time requirements, the difference in reaction time between the first and last droplet applied is insignificant.

#### EXAMPLE 3

##### Construction of Piezoelectric Impulse Jet Pump Apparatus

Piezoelectric impulse jets are fabricated from Photoceram (Corning Glass, Corning, N.Y.), a UV sensitive ceramic, using standard photolithographic techniques to produce the pump details. The ceramic is fired to convert it to a glassy state. The resulting blank is then etched by hydrogen fluoride, which acts faster in exposed than in nonexposed areas. After the cavity and nozzle details are lapped to the appropriate thickness in one plate, the completed chamber is formed by diffusion bonding a second (top) plate to the first plate. The nozzle face is lapped flat and surface treated, then the piezoelectric element is epoxied to the outside of the pumping chamber. When the piezoelectric element is energized it deforms the cavity much like a one-sided bellows, as shown in FIG. 5.

To determine the appropriate orifice size for accurate firing of acetonitrile droplets, a jet head with a series of decreasing orifice sizes is prepared and tested. A 40 micron nozzle produces droplets of about 65 picoliter.

A separate nozzle array head is provided for each of the four nucleotides and a fifth head is provided to deliver the activating reagent for coupling. The five heads are stacked together with a mechanically defined spacing. Each head has an array of eight nozzles with a separation of 400 microns.

The completed pump unit is assembled with the heads held stationary and the droplets fired downward at a moving array plate as shown in FIG. 6. The completed pump unit assembly (3) consists of nozzle array heads (4-7) for each of the four nucleotidase and a fifth head (8) for activating reagent. When energized, a microdroplet (9) is ejected from the pump nozzle and deposited on the array plate (1) at a functionalized binding site (2).

A plate holding the target array is held in a mechanical stage and is indexed in the X and Y planes beneath the heads by a synchronous screw drives. The mechanical stage is similar to those used in small milling machines, microscopes and microtomes, and provides reproducible positioning accuracy better than 2.5 microns or 0.1 mil. As shown in FIG. 7, the plate holder (3) is fitted with a slotted spacer (4) which permits a cover plate (5) to be slid over the array (6) to form an enclosed chamber. Peripheral inlet (1) and outlet (2) ports are provided to allow the plate to be flooded for washing, application of reagents for a common array reaction, or blowing the plate dry for the next dot array application cycle.

Both the stage and head assembly are enclosed in a glove box which can be evacuated or purged with argon to maintain anhydrous conditions. With the plate holder slid out of the way, the inlet lines to the heads can be pressurized for positive displacement priming of the head chambers or flushing with clean solvent. During operation, the reagent vials are maintained at the ambient pressure of the box.

With a six minute chemistry cycle time, the apparatus can produce 10-mer array plates at the rate of 1 plate or 106 oligonucleotides per hour.

#### EXAMPLE 4

##### Use of Oligonucleotide Array Plates to Determine the Nucleotide Sequence of a Target Nucleic Acid

The oligonucleotide array plate is prepared as described in Examples 1 and 2, using the apparatus described in Example 3. The array contains oligonucleotides having 10 nucleotides each (10-mers). The synthesis is carried out such that each oligonucleotide element, moving in a 5'-3' direction, is identical to the preceding element in nucleotide sequence, except that it deletes the 5'-most nucleotide, and adds a new 3'-most oligonucleotide. In this way the total array represents every possible permutation of the 10-mer oligonucleotide. Oligonucleotides are spaced at 7 nm intervals to provide an oligonucleotide loading density of  $3.4 \times 10^{-12}$  moles/cm<sup>2</sup>, or  $2.6 \times 10^{-16}$  moles per 100 micron element. The target nucleic acid is used to probe the oligonucleotide array plate. The probe is labelled with 1000 Ci/nmol p<sup>32</sup>. The labelled probe is contacted with the oligonucleotide array plate for hybridization in a 10 nM solution of probe in 3M Me<sub>4</sub>NCl at 42° C. At 10% hybridization and wash efficiency, each oligonucleotide element dot having an exact match with the probe binds 26 attomoles of probe. Radiolabel binding is detected using a Bio-Image Analyzer™ (Fuji, Waltham, Mass.). The pattern of binding is assessed and the nucleotide sequence of the probe nucleic acid is determined by ordering the nucleotide sequence according to the known sequences of the oligonucleotide elements, as shown in FIG. 1.

FIG. 1 depicts a sequencing arrangement based on a matrix of trimer oligonucleotides bound to the array plate. FIG. 1(a) is the basic matrix consisting of the four nucleotides. FIG. 1(b) is the complete trimer matrix, representing each of the 43 trimer permutations. The underlined elements in the array represent sites to which the target nucleic acid is bound. FIG. 1(c) depicts how a sequence complementary to the target nucleic acid is constructed from the known sequences of the sites to which the target nucleic acid is bound.

What is claimed is:

1. An array plate comprising a support surface comprising a covalently linked layer of inert siloxane, wherein said covalently linked layer defines an array of 10 to 10<sup>4</sup> sites per cm<sup>2</sup>, which do not have said covalently linked layer, and wherein chemical reactant solutions localize to said sites via surface tension.
2. The array plate according to claim 1, wherein said siloxane is tridecafluoro-1,1,2,2-tetrahydrooctyl siloxane.
3. The array plate according to claim 1, wherein said sites are functionalized to bind or covalently link a nucleic acid.
4. The array plate according to claim 3, wherein said sites comprise siloxane compounds selected from the group con-

sisting of hydroxyalkyl siloxane, dihydroxyalkyl siloxanes, and aminoalkyl siloxanes.

5. A method of using an array plate comprising a support surface comprising a covalently linked layer of inert siloxane, wherein said covalently linked layer defines an array of 10 to 10<sup>4</sup> sites per cm<sup>2</sup>, which do not have said covalently linked layer, and wherein chemical reactant solutions localize to said sites via surface tension, said method comprising depositing a solution of nucleic acid reagent on said array plate in an amount such that said solution of nucleic acid reagent at said sites stays separated from said solution of nucleic acid reagent at other sites due to surface tension.

6. A method of using an array plate for conducting nucleic acid reactions, wherein said array plate comprises a support surface comprising a covalently linked layer of inert siloxane, said covalently linked layer defines an array of 10 to 10<sup>4</sup> functionalized binding sites per cm<sup>2</sup>, which do not have said covalently linked layer, such that chemical reactant solutions localize to said sites via surface tension, said method comprising depositing a solution of nucleic acid reagent on said array plate to chemically react with said functionalized binding sites in an amount such that said solution of nucleic acid reagent at each said binding site stays separated from said solution of nucleic acid reagent at other sites due to surface tension.

7. The method according to claim 5 or 6, wherein said depositing is performed using an ink jet printing apparatus.

8. The method according to claim 5 or 6, wherein said depositing is performed using a piezoelectric pump.

9. The method according to claim 5 or 6, wherein said amount is about 50 picoliters to about 2 microliters.

10. A method according to claim 6, wherein said nucleic acid reactions form covalent bonds between said nucleic acid reagent and said functionalized binding sites.

11. A method of using an array plate for conducting nucleic acid reactions, wherein said array plate comprises a support surface comprising a covalently linked layer of inert siloxane, said covalently linked layer defines an array of 10 to 10<sup>4</sup> functionalized binding sites per cm<sup>2</sup>, which do not have said covalently linked layer, and said functionalized binding sites are more polar than the surrounding surface, said method comprising depositing a solution of nucleic acid reagent on said functionalized binding sites in an amount such that said solution of nucleic acid reagent at each of said binding sites stays separated from said solution of nucleic acid reagent at other sites due to differential wetting of said more polar functionalized binding sites.

12. The method according to claim 11, wherein said depositing is performed using an ink jet printer apparatus.

13. The method according to claim 11, wherein said depositing is performed using a piezoelectric pump.

14. The method according to claim 11, wherein said amount is about 50 picoliters to about 2 microliters.

15. A method according to claim 11, wherein said nucleic acid reaction forms covalent bonds between said nucleic acid reagent and said functionalized binding site.

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**(12) United States Patent**  
Becker et al.**(10) Patent No.: US 6,225,061 B1**  
**(45) Date of Patent: May 1, 2001****(54) SYSTEMS AND METHODS FOR  
PERFORMING REACTIONS IN AN  
UNSEALED ENVIRONMENT****(75) Inventors:** Thomas Becker, Lineberg (DE);  
Hubert Köster, La Jolla, CA (US);  
Charles Cantor, Boston, MA (US)**(73) Assignee:** Sequenom, Inc., San Diego, CA (US)**(\*) Notice:** Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.**(21) Appl. No.:** 09/266,409**(22) Filed:** Mar. 10, 1999**(51) Int. Cl.<sup>7</sup>** ..... C12Q 1/68**(52) U.S. Cl.** ..... 435/6; 435/6; 435/177;  
159/1; 159/47.1; 422/63; 422/64; 422/65;  
422/66; 422/67; 436/177; 436/43  
**(58) Field of Search** ..... 435/6, 177; 159/1,  
159/47.1; 422/63, 64, 65, 66, 67; 436/177,  
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**Primary Examiner**—W. Gary Jones**Assistant Examiner**—Janell E. Taylor**(74) Attorney, Agent, or Firm**—Stephanie L. Seidman;  
Heller Ehrman White & McAuliffe LLP**(57) ABSTRACT**

An open system is provided for performing a submicroliter reaction. An open system can contain a solid support having a target site for performing the reaction; a liquid dispensing system such as a nanoliter dispensing pipette for dispensing a submicroliter amount of a liquid to the target site; a temperature controlling device for regulating the temperature of the support; and means for controlling the amount of liquid dispensed, which corresponds to the amount of liquid that evaporates from the target site. Also provided is an open system, including a solid support having a target site; a liquid dispensing system, which can dispense a liquid to the target site; a temperature controlling system, which regulates the temperature of the solid support; and an interface, which regulates an amount of liquid dispensed from the liquid dispensing system. Also provided is a method for performing a reaction in a submicroliter volume in an unsealed environment by dispensing a submicroliter volume of liquid onto the surface of a support; monitoring the temperature of the support; monitoring an amount or rate of evaporation of the liquid; and dispensing to the surface of the support a further amount of the liquid, which corresponds to the amount lost from the support due to evaporation, thereby maintaining the reaction volume at a predetermined volume throughout the course of the reaction. A method also is provided for maintaining a volume of a reaction mixture, which can be one of a plurality of reaction mixtures, on a solid support in an unsealed environment by monitoring the rate of evaporation of a liquid from the reaction mixture; and dispensing into the reaction mixture an amount of liquid that corresponds to the amount that evaporates.

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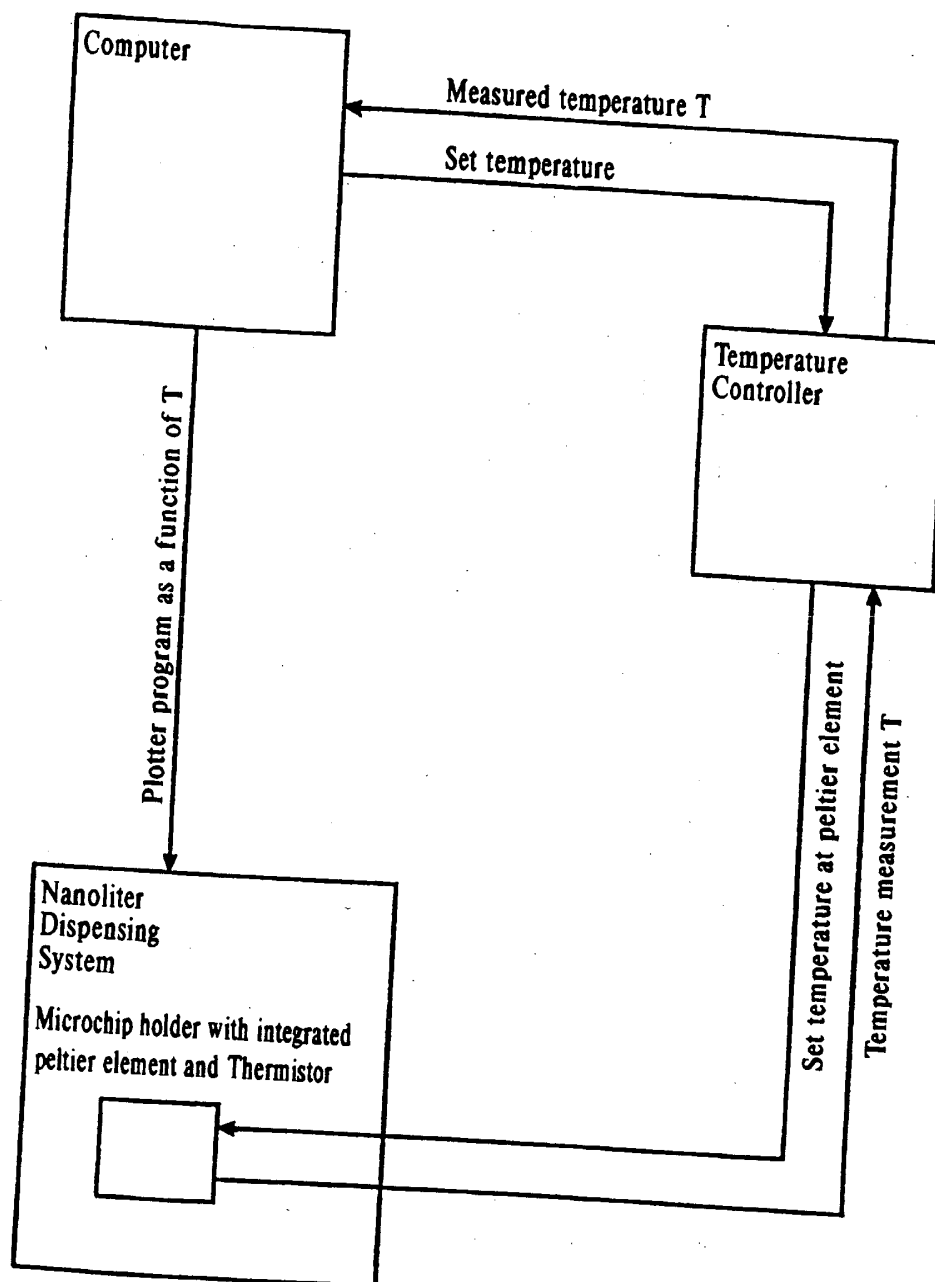


FIG. 1

# SYSTEMS AND METHODS FOR PERFORMING REACTIONS IN AN UNSEALED ENVIRONMENT

## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

The invention relates generally to systems and methods for performing a reaction in a small volume without incurring an undesirable loss of the reaction volume due to evaporation, and more specifically to systems and methods for performing reactions involving polymers, particularly biopolymers, in a reaction volume of a few microliters or less in an unsealed environment.

### 2. Background Information

Technological advances have allowed an examination of previously undiscernible phenomena. Such advances are particularly notable in the biological sciences, where the chemical and physical structures of many biopolymers have been described, and where such biopolymers, including DNA and proteins, routinely are synthesized and sequenced.

Although methods such as nucleic acid sequencing and synthesis have contributed to understanding the structure and function of biological molecules and their relationships to disease, the limitations of such methods are apparent. For example, it generally is agreed that knowledge of the entire sequence of the human genome would provide valuable insight into the prevention and treatment of disease. The human genome, however, contains over one billion nucleotides and a huge expenditure of labor and money would be required to sequence the entire human genome. Furthermore, using currently available methods, many years will be required to see the project to completion.

Similarly, it is a goal of most clinical researchers to develop rapid and simple tests for determining whether an individual has a disease or predisposition to a disease. In many cases, the signs and symptoms of many genetic diseases do not become apparent until an individual reaches a certain age or stage of development. Knowledge that an individual has a predisposition to a genetic disease can allow the clinician to take prophylactic measures to minimize or delay onset of the disease. Ideally, all individuals would be screened for potential genetically determined diseases, including screening a large number of genes in each individual. Unfortunately, such routine screening currently is not feasible because the assays are time consuming and the reagents for performing such assays are expensive and limited in availability.

In an effort to reduce the time and cost for analyzing biopolymers, including genes and proteins, processes are being developed to automate the analytic procedures. Automation provides a means for performing repetitive processes almost continually, except for periodic breaks for equipment maintenance, and allows researchers and technical staff to devote more time to other endeavors, including interpreting the results produced by the automated assays and troubleshooting problems that may arise. Automation of repetitive processes also provides the advantage that the likelihood of errors occurring, for example, due to fatigue or distraction is reduced and, therefore, more accurate results can be obtained.

The application of nanotechnology to the biological sciences promises to provide the next breakthroughs relating, for example, to the analysis, synthesis and utilization of biopolymers. Nanotechnology, which provides processes and apparatuses for performing procedures on a very small

scale, has been developed by the semiconductor industry in order to produce smaller and smaller microchips, and to allow placement of a continually increasing number of instructions on a microchip.

Efforts are in progress to apply nanotechnology to chemical and biological procedures, thereby providing a means to perform assays in very small volumes, generally a few hundred nanoliters or less. Application of nanotechnology to biological assays can be particularly valuable because a critical limitation of many biological assays is the amount of biological material available for analysis. By performing such assays in nanoliter volumes or smaller, the effective concentration of a biopolymer in a biological reaction is increased, thereby providing the necessary kinetics for a biological reaction to proceed. In addition, the ability to perform biological assays in nanoliter volumes can provide a significant cost savings because much smaller amounts of reagents, which can be very expensive, can be utilized in the reactions.

The application of nanotechnology to biological assays has been hindered, in part, by the difficulty in manipulating and maintaining such small volumes. Many biological assays, for example, are performed in aqueous conditions, using water as a solvent, and at elevated temperatures, generally at least 37° C., which is human body temperature. Water, like many liquid solvents, is susceptible to evaporation and, therefore, as the time or temperature of a reaction increases, the loss of water due to evaporation increases and the volume of the reaction decreases. As a result of evaporation, the effective concentration of reagents in the reaction increases, thereby changing the conditions of the reaction. Since most biological assays are quite sensitive to reaction conditions, loss of water or other solvent from a reaction can result in an assay that produces spurious results. Any loss of a solvent such as water is particularly deleterious when the reaction contains only a few hundred nanoliters or less of the liquid, since the reaction quickly can evaporate to dryness.

Various methods have been used to minimize the loss of solvent in a reaction due to evaporation in biochemical assays. For example, reaction mixtures can be drawn into glass capillary tubes, which then are sealed at both ends for the reaction. Small volume glass capillary tubes can be expensive, and the use of such tubes requires additional steps, including sealing and unsealing the tube, the latter which can produce glass shards.

In many cases, reaction mixtures are performed in a microcentrifuge tube or other open chamber, and evaporation is minimized by overlaying the reaction mixture with wax, mineral oil, or other nonvolatile compound during the reaction. Such a method, again, requires additional steps, including removing the sealing material following the reaction. In order to remove all or most of the sealing material, which can otherwise contaminate the sample and hinder further analysis, some loss of the sample being assayed inevitably occurs. Since most biological samples are limited to begin with, any loss of sample can preclude an interpretation of the results of the assay. In general, any additional manipulations of a sample will incur extra cost, either in terms of time or money, and loss or contamination of the sample.

More recently, biological reactions have been performed on microchips, which conveniently can be adapted to automated processes. Such microchips have been designed having a system including, for example, chambers, which hold the reactants, and channels, which connect the chambers and

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in which the reactants can be mixed and a reaction performed. Since the channels, in which the reaction occurs, provide a sealed or closed environment, there is little or no evaporative loss of the reaction volume. Thus far, however, the technology for preparing such a device allows for the placement of only one or few of such closed systems on a single microchip and, therefore, the number of reactions that can be performed at one time on a single chip is limited. Thus, a need exists for systems useful for performing reactions in a volume of a few microliters or less in an unsealed environment. Therefore it is an object here to provide systems and methods that satisfy this need and also provide additional advantages.

### SUMMARY OF THE INVENTION

Systems are provided for performing a reaction in an unsealed environment. The systems are used for any desired reaction, including, but not limited to in situ biopolymer or polymer synthesis, such as nucleic acid and protein syntheses, protein and nucleic acid sequencing methods, such as oligonucleotide-based primer extension, nucleic acid amplification reactions, protein and nucleic acid protease- or nuclease-based degradations and others.

A system as disclosed herein is an open system for performing a reaction, such as a synthetic reaction or an assay, particularly in a submicroliter volume. The systems can include a support for performing the reaction; a nanoliter dispensing pipette for dispensing a submicroliter amount of a liquid to a target site on the support; a temperature controlling device for regulating the temperature of the surface of the support; and means for controlling the amount of liquid dispensed, where the amount of liquid dispensed corresponds to the amount of liquid evaporated from the support. A means for controlling the amount of liquid dispensed can include computer software that calculates the rate of evaporation and signals the dispensing pipette to deliver an amount of the liquid that corresponds to the amount lost due to evaporation. A means for controlling the amount of liquid dispensed also can be manual input, which can be performed by an individual.

A system as disclosed herein also can include a temperature measuring device for measuring the temperature of the surface of the support. The support can be any support having a surface, including, for example, a bead, pin, comb, wafer, well or microchip, and the support can be functionalized such that a substrate, for example, a biopolymer can be linked, either directly or indirectly via covalent or non-covalent interactions, to the support and immobilized.

An open system, as disclosed herein, also can include a solid support, which has a target site that can contain a volume of liquid, for example, a reaction mixture; a liquid dispensing system, which can dispense a liquid to the target site; a temperature controlling system, which can regulate the temperature of the solid support; and an interface, which can indicate an amount of liquid to be dispensed to the target site from the liquid dispensing system. An interface can include, for example, a computer using an appropriate algorithm. A computer can monitor the temperature of the solid support and, based on various parameters, including, for example, the chemical nature of the liquid, the surface area of the liquid exposed to the environment, and the time the liquid is maintained at a particular temperature, and can provide information as to the amount of liquid to be dispensed from the liquid dispensing system to the target site to maintain the liquid at a predetermined volume. Based on that information, the liquid dispensing system can be manipu-

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lated manually, to dispense the liquid to the target site, or can be controlled automatically, for example, by interfacing it with the computer. In a system as disclosed herein, the amount of liquid dispensed from a liquid dispensing system to a target site generally corresponds to an amount of liquid lost from the target site due to evaporation, although the amount added also can be an initial amount added to a target site or an amount added to modify the conditions of a reaction.

An open system, as disclosed herein, also can include a solid support having a target site; a liquid dispensing system, which can dispense a liquid to the target site; a temperature controlling system, which regulates the temperature of the solid support; and means for regulating an amount of liquid dispensed from the liquid dispensing system. In addition, an open system, as disclosed herein, can have a means for containing a reaction mixture; a means for dispensing a liquid; a means for controlling the temperature of the reaction volume containing means; and means for regulating an amount of liquid dispensed from the liquid dispensing means.

A means for regulating an amount of liquid dispensed can be a computer having an appropriate algorithm. Such a computer can interface with the solid support, thereby monitoring the temperature of the support, and can provide an indication of an amount of liquid to be dispensed to a target site to maintain a predetermined volume, for example, of a reaction volume. The computer can cause to be displayed the amount of liquid to be dispensed, such that an individual can manipulate the liquid dispensing system and dispense the liquid, or the computer can further interface with the liquid dispensing system, thereby causing the amount of liquid to be dispensed. In addition, charts can be developed that predict the amount and rate of evaporation of a particular solvent at a particular temperature and, based on such charts, an individual can manipulate the liquid dispensing system as necessary. Also, a decrease in the volume of a liquid due to evaporation can be identified directly by including the liquid in a circuit, wherein, when the liquid falls below a predetermined point, the circuit is broken, thereby indicating that a liquid should be dispensed to the target site until the circuit is reestablished.

Methods for maintaining a volume of a liquid in an unsealed environment also are provided. A method for performing a reaction in a predetermined submicroliter volume in the open can be performed by dispensing the predetermined submicroliter volume of liquid onto the surface of a support; optionally monitoring the temperature of the substrate; determining the amount or rate of evaporation of the liquid from the support; and dispensing a further amount of the liquid to the surface of the support, wherein the further amount dispensed corresponds to the amount lost from the support due to evaporation, thereby maintaining the reaction volume at a predetermined volume throughout the course of the reaction. Such a method also can be performed, for example, by determining the temperature of a solid support, which has a target site that can contain a volume of liquid; and, based on the temperature, dispensing at the target site an amount of liquid required to maintain a predetermined volume of the liquid. The amount of liquid to be dispensed can be determined using a computer algorithm, which, based on various parameters, including the temperature of the support, the chemical nature of the liquid, the surface area of the liquid exposed to the environment, and the volume to be maintained, can indicate the amount of liquid that evaporates from the site and, therefore, the amount of liquid to be dispensed to maintain a predeter-



mined volume. The volume of a liquid on a target site also can be monitored, for example, by microscopic examination, using an appropriate optical system or a video imaging device, such that, as the volume of a liquid at a target site decreases due to evaporation, a corresponding amount of liquid can be dispensed to maintain the volume within acceptable parameters.

Methods for performing a reaction in an unsealed environment also are provided. Such a method can be performed, for example, by determining the temperature of a solid support, which has a target site containing a volume of the reaction mixture, or determining the rate or amount of evaporation of liquid from the reaction mixture; and dispensing into the reaction mixture an amount of liquid required to maintain the volume at a predetermined level. Such a method is particularly useful where the reaction mixture has a volume of a few microliters or less, particularly a volume of about 500 nanoliters or less. The disclosed methods also are useful for performing submicroliter reactions at temperatures where the vapor pressure of a liquid in the reaction mixture is undesirably high, for example, about 2.5 kilopascals (kPa) or greater, particularly about 5 kPa or greater, or about 10 kPa or greater, such that evaporation of the liquid can substantially change the volume of the reaction mixture and adversely affect the reaction. As such, the disclosed methods are useful for performing various chemical, physical and biological reactions, for example, a polymerase chain reaction, or a nucleic acid or polypeptide synthesis or sequencing reaction or other reaction or assay performed on a solid support.

#### BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 provides an exemplary embodiment of an open system for performing a reaction in an unsealed environment. A liquid is contained on a target site on the surface of a microchip, which is in a holder that is integrated with a Peltier element and thermistor. The temperature of the Peltier element/thermistor is regulated by a temperature controller, which is interfaced with a computer, and the temperature of the chip is monitored. The computer, which also is interfaced with the nanoliter dispensing device, receives input of the measured temperature, calculates the amount of liquid that evaporates from the target site, and signals the dispensing device to dispense an amount of liquid that corresponds to the amount that evaporates, thereby maintaining the volume of liquid on the target site at a predetermined level.

#### DETAILED DESCRIPTION OF THE INVENTION

A system is provided for performing reactions in an unsealed environment, including reactions performed in submicroliter volumes. An open system as disclosed herein solves the previously intractable problems caused by evaporation of a liquid solvent during a reaction, including, for example, the concomitant increase in the effective salt concentration, which can inhibit a reaction or lead to spurious results. An additional advantage of the disclosed open systems and methods of performing a reaction in an unsealed environment is that fewer steps are required to perform a reaction because the reaction volume need not be sealed or covered with a protective layer such as mineral oil to prevent evaporation, such manipulations further requiring that the volume later be unsealed or isolated from the protective layer. As a result, the likelihood that any sample will be lost due to the additional manipulations is reduced.

An open system as disclosed herein provides the further advantage that "single tube" reactions can be performed, wherein a number of different reactions are performed at the same target site. The ability to perform single tube reactions further reduces the likelihood that any sample will be lost due to transferring a material from one tube to another for performing different reactions, and facilitates the automation of chemical and biological reactions. It should be recognized that such reactions, while referred to as "single tube" reactions, need not literally be performed in a "tube," but can be performed at any target site having the characteristics disclosed herein.

The disclosed open systems are useful for performing liquid handling, for example, for performing reactions such as polymerase chain reaction (PCR), DNA sequencing and enzymatic digestion reactions. Such reactions can be performed directly on the surface of a modified silicon chip that can be used for mass spectrometric detection of the resulting products, or allows on-line monitoring of fluorescent or luminescent signals. It is important to prevent evaporation of a solvent, generally water, of a reaction to prevent alterations in concentrations of reactants or other components such as salts during the reactions. Typically, evaporation is prevented by performing reactions in sealed or closed environments. The methods and systems disclosed herein permit reactions to be performed, for example, directly on the surface of a microchip without a need for a lid or sealing. This achieved by replacing nanoliter amounts of water or water/glycerol or other reaction mixture components in the reaction mixture using drop-on-demand systems, which compensate for loss of solvent by evaporation.

An open system as disclosed herein can include a support for performing the reaction; a nanoliter dispensing pipette for dispensing a submicroliter amount of a liquid onto the surface of the support; a temperature controlling device for regulating the temperature of a target site on the support, particularly of a liquid at the target site; and means for controlling the amount of liquid dispensed, wherein the amount of liquid dispensed corresponds to the amount of evaporation of a the liquid from the support. A system as disclosed herein also can include a temperature measuring device for measuring the temperature of the surface of the support. The support can be any support having a surface, including, for example, a bead, pin, comb, wafer, well or microchip, and the support can be functionalized such that a biopolymer can be linked to the support and immobilized.

A means for controlling the amount of liquid dispensed can include computer software that calculates the rate or amount of evaporation of the liquid and signals the dispensing pipette to deliver an amount of the liquid that corresponds to the amount lost due to evaporation. A means for controlling the amount of liquid dispensed also can be manual input, which can be performed by an individual. In addition, a means for controlling the amount of liquid dispensed can be a system that determines when a meniscus of a liquid decreases below a predetermined point. Such a system can be, for example, an electrical circuit, which is broken when the meniscus falls below a predetermined point; or a photometric or spectrophotometric system, which detects a change in diffraction, transmission or absorbance of photons when the meniscus falls below a predetermined point. Such a meniscus determining means conveniently can provide an interface between the target site and the liquid dispensing system. A means for controlling the amount of liquid dispensed also can be a system for determining the conductivity (or resistivity) of the liquid, which changes in parallel with a change in the reaction volume, such that,

when the conductivity (or resistivity) reaches a predetermined value, an indication is provided as to an amount of liquid to be dispensed to the target site to maintain the liquid at a predetermined volume.

An open system as disclosed allows a reaction to be performed in an unsealed environment. An open system can include a solid support, which has a target site that contains the reaction mixture; a liquid dispensing system; a temperature controlling system, which regulates the temperature of the solid support; and an interface that regulates an amount of liquid dispensed from the liquid dispensing system. The interface can indicate an amount of liquid to be dispensed based, for example, on the temperature of the solid support or the decrease of a meniscus below a predetermined point, and the amount of liquid dispensed can correlate with the amount of liquid that evaporates from a reaction mixture on the solid support. Also provided is a system having means for dispensing a liquid; means for containing a reaction volume; means for controlling the temperature of the reaction volume containing means; and means for regulating an amount of liquid dispensed from the liquid dispensing means based on the temperature of the reaction volume containing means. A means for containing a reaction volume can be a solid support having, for example, a well or pin, or a barrier, which can be a physical or chemical barrier.

#### Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents, pending and published applications and publications referred to herein are incorporated by reference.

As used herein, the term "unsealed environment," when used in reference to a volume of a liquid, means that there is no particular barrier present to prevent substantial evaporation of the liquid into the environment. For purposes herein, substantial evaporation occurs when evaporation occurs at a rate or amount that alters the reaction conditions before the reaction of interest is completed. This is particularly problematic for reactions that are performed in wells or on the surface of solids small volumes, typically submicroliter volumes. Hence, methods and systems are provided herein to permit such reactions to be performed.

As used herein, an open system refers to the systems disclosed herein for maintaining a volume of a liquid in an unsealed environment. These can be referred to generally as "open systems" although such systems can be sealed from the air, such as under inert gas or in a box or other container. As noted an open system is one in which evaporation occurs during the reaction of interest in an amount or rate such that conditions of the reaction are altered, such by a change in concentration of critical components, such as salt concentrations. This will occur if small volume, such as submicroliter reactions, are performed in wells or on the surface of a solid support. The disclosed systems and methods, thus, are distinguishable from systems and methods for performing a reaction, for example, in an unsealed microcapillary tube or in a channel on a microchip because, even though a liquid may be in direct contact to the open air in such systems, the amount of evaporation that occurs is not unacceptable either because the evaporation is desired, or because the surface area of the liquid in contact with the gaseous medium is so small with respect to the volume of the liquid that any evaporation that occurs during the reaction period does not deleteriously affect or alter the reaction conditions.

As used herein, the term "liquid dispensing system" means a device that can transfer a predetermined amount of liquid to a target site. The amount of liquid dispensed and the

rate at which the liquid dispensing system dispenses the liquid to a target site, which can contain a reaction mixture, can be adjusted manually or automatically, thereby allowing a predetermined volume of the liquid to be maintained at the target site.

As used herein, the term "liquid" is used broadly to mean a non-solid, non-gaseous material, which can be homogeneous or heterogeneous and can contain one or more solid or gaseous materials dissolved or suspended therein. In general, a liquid is a component of a reaction mixture that is susceptible to evaporation under the conditions of the reaction. In particular, the liquid can be a solvent, in which a reaction is performed, for example water or glycerol/water or buffer or reaction mixture, where the reaction is performed in an aqueous solution. The liquid can be any non-solid, non-gaseous solvent or other component of a reaction mixture that is susceptible to evaporative loss, for example, acetonitrile, which can be a solvent for a nucleic acid synthesis reaction; formamide, which can be a liquid component of a nucleic acid hybridization reaction; piperidine, which is a liquid component of a nucleic acid sequencing reaction; or any other non-aqueous solvent or other liquid component. A liquid can contain dissolved or suspended components, which can be useful, for example, for initiating, terminating or changing the conditions of a reaction, thereby facilitating the performance of single tube reactions.

As used herein, the term "reaction mixture" refers to any solution in which a chemical, physical or biological change is effected. In general, a change to a molecule is effected, although changes to cells also are contemplated. A reaction mixture can contain a solvent, which provides, in part, appropriate conditions for the change to be effected, and a substrate, upon which the change is effected. A reaction mixture also can contain various reagents, including buffers, salts, and metal cofactors, and can contain reagents specific to a reaction, for example, enzymes, nucleoside triphosphates, amino acids, and the like. For convenience, reference is made herein generally to a "component" of a reaction, wherein the component can be a cell or molecule present in a reaction mixture, including, for example, a biopolymer or a product thereof.

As used herein, the term "biopolymer" is used to mean a biological molecule composed of two or more monomeric subunits, or derivatives thereof, which are linked by a bond or a macromolecule. A biopolymer can be, for example, a polynucleotide, a polypeptide, a carbohydrate, or a lipid, or derivatives or combinations thereof, for example, a nucleic acid molecule containing a peptide nucleic acid portion or a glycoprotein, respectively. The methods and systems herein, though described with reference to biopolymers, can be adapted for use with other synthetic schemes and assays, such as organic syntheses of pharmaceuticals, or inorganics and any other reaction or assay performed on a solid support or in a well in nanoliter volumes.

As used herein, a biological particle refers to a virus, such as a viral vector or viral capsid without or without packaged nucleic acid, phage, including a phage vector or phage capsid, with or without encapsulated nucleotide acid, a single cell, including eukaryotic and prokaryotic cells or fragments thereof, and other such biological materials.

As used herein, the term "polynucleotide" refers to an oligomer or polymer containing at least two linked nucleotides or nucleotide derivatives, including a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), and a DNA or RNA derivative containing, for example, a nucleotide analog or a "backbone" bond other than a phosphodiester

bond, for example, a phosphotriester bond, a phosphoramidate bond, a phosphorothioate bond, a thioester bond, or a peptide bond (peptide nucleic acid). The term "oligonucleotide" also is used herein essentially synonymously with "polynucleotide," although those in the art will recognize that oligonucleotides, for example, PCR primers, generally are less than about fifty to one hundred nucleotides in length.

Nucleotide analogs contained in a polynucleotide can be, for example, mass modified nucleotides, which allows for mass differentiation of polynucleotides; nucleotides containing a detectable label such as a fluorescent, radioactive, luminescent or chemiluminescent label, which allows for detection of a polynucleotide; or nucleotides containing a reactive group such as biotin or a thiol group, which facilitates immobilization of a polynucleotide to a solid support. A polynucleotide also can contain one or more backbone bonds that are selectively cleavable, for example, chemically, enzymatically or photolytically. For example, a polynucleotide can include one or more deoxyribonucleotides, followed by one or more ribonucleotides, which can be followed by one or more deoxyribonucleotides, such a sequence being cleavable at the ribonucleotide sequence by base hydrolysis. A polynucleotide also can contain one or more bonds that are relatively resistant to cleavage, for example, a chimeric oligonucleotide primer, which can include nucleotides linked by peptide nucleic acid bonds and at least one nucleotide at the 3' end, which is linked by a phosphodiester bond, or the like, and is capable of being extended by a polymerase. Peptide nucleic acid sequences can be prepared using well known methods (see, for example, Weiler et al., *Nucleic acids Res.* 25:2792-2799 (1997)).

A polynucleotide can be a portion of a larger nucleic acid molecule, for example, a portion of a gene, which can contain a polymorphic region, or a portion of an extragenic region of a chromosome, for example, a portion of a region of nucleotide repeats such as a short tandem repeat (STR) locus, a variable number of tandem repeats (VNTR) locus, a microsatellite locus or a minisatellite locus. A polynucleotide also can be single stranded or double stranded, including, for example, a DNA-RNA hybrid, or can be triple stranded or four stranded. Where the polynucleotide is double stranded DNA, it can be in an A, B, L or Z configuration, and a single polynucleotide can contain combinations of such configurations.

As used herein, the term "polypeptide," means at least two amino acids, or amino acid derivatives, including mass modified amino acids and amino acid analogs, that are linked by a peptide bond, which can be a modified peptide bond. A polypeptide can be translated from a polynucleotide, which can include at least a portion of a coding sequence, or a portion of a nucleotide sequence that is not naturally translated due, for example, to it being located in a reading frame other than a coding frame, or it being an intron sequence, a 3' or 5' untranslated sequence, a regulatory sequence such as a promoter, or the like. A polypeptide also can be chemically synthesized and can be modified by chemical or enzymatic methods following translation or chemical synthesis. The terms "polypeptide," "peptide" and "protein" are used essentially synonymously herein, although the skilled artisan will recognize that peptides generally contain fewer than about fifty to one hundred amino acid residues, and that proteins often are obtained from a natural source and can contain, for example, post-translational modifications. A polypeptide can be post-translationally modified by phosphorylation (phosphoproteins), glycosylation (glycoproteins,

proteoglycans), and the like, which can be performed in a cell or in a reaction in vitro.

As used herein, a reaction mixture used in an open system or method as disclosed herein can have any volume from a few picoliters or less to hundreds of liters or more. An open system or method as disclosed herein is particularly useful where a volume to be maintained is critical in order for a reaction to occur, and where the volume to be maintained is not amenable to simple inspection or measurement. As such, the disclosed systems and methods generally are useful where the reaction volume is about 500 milliliters or less; are more useful where the reaction volume is about 5 milliliters or less; are most useful where the reaction volume is in the "submilliliter" range, for example, about 500 microliters, or about 50 microliters or about 5 microliters or less; and are particularly useful where the reaction volume is a "submicroliter" reaction volume, which can be measured in nanoliters, for example, about 500 nanoliters or less, or 50 nanoliters or less or 10 nanoliters or less, or can be measured in picoliters, for example, about 500 picoliters or less or about 50 picoliters or less. For convenience of discussion, the term "submicroliter" is used herein to refer to a reaction volume less than about one microliter, although it will be readily apparent to those in the art that the systems and methods disclosed herein are applicable to subnanoliter reaction volumes as well. A reaction mixture is contained in or on a target site on a solid support.

As used herein, the term "solid support" means a non-gaseous, non-liquid material having a surface. Thus, a solid support can be a flat surface constructed, for example, of glass, silicon, metal, plastic or a composite; or can be in the form of a bead such as a silica gel, a controlled pore glass, a magnetic or cellulose bead; or can be a pin, including an array of pins suitable for combinatorial synthesis or analysis.

As used herein, the term "target site" refers to a specific locus on a solid support that can contain a liquid. A solid support contains one or more target sites, which can be arranged randomly or in ordered array or other pattern. In particular, a target site restricts growth of a liquid to the "z" direction of an xyz coordinate. Thus, a target site can be, for example, a well or pit, a pin or bead, or a physical barrier that is positioned on a surface of the solid support, or combinations thereof such as a beads on a chip, chips in wells, or the like. A target site can be physically placed onto the support, can be etched on a surface of the support, can be a "tower" that remains following etching around a locus, or can be defined by physico-chemical parameters such as relative hydrophilicity, hydrophobicity, or any other surface chemistry that allows a liquid to grow primarily in the z direction. A solid support can have a single target site, or can contain a number of target sites, which can be the same or different, and where the solid support contains more than one target site, the target sites can be arranged in any pattern, including, for example, an array, in which the location of each target site is defined.

As used herein, the term "predetermined volume" is used to mean any desired volume of a liquid. For example, where it is desirable to perform a reaction in a 5 microliter volume, 5 microliters is the predetermined volume. Similarly, where it is desired to deposit 200 nanoliters at a target site, 200 nanoliters is the predetermined volume.

As used herein, the term "maintain a volume of a liquid" refers to a predetermined volume of the liquid and means that the volume of the liquid is kept within an acceptable amount of the predetermined volume. An acceptable amount of a predetermined volume is an amount that is within 80% or more of the predetermined volume, generally within

about 90% or more, and particularly about 95%, or about 98% or more of the predetermined volume.

As used herein, a volume of a liquid at a target site is maintained within a predetermined volume by dispensing an amount of liquid to the target site. In one embodiment, the amount of liquid dispensed to a target site is based on the evaporation rate of the liquid from the target site, such that the amount of liquid dispensed corresponds to the amount of liquid lost from the volume due to evaporation. As used herein, the term "corresponds," when used in reference to the amount of liquid being added to the reaction mixture ("amount added") and the amount of liquid that evaporates from the reaction mixture ("amount lost"), means that the amount added is, within an acceptable margin of error, equal to the amount lost. An acceptable margin of error is such that the amount added is within 20% or less of the amount lost, generally within about 10% or less, and particularly about 5% or less, or about 2% or less of the amount lost. An acceptable margin of error can be determined based, for example, on the susceptibility of a reaction to the effective concentration of one or more reactants in the reaction. In another embodiment, the disclosed systems and methods allow a predetermined amount of a liquid to be dispensed to a target site, for example, to initiate a reaction, to dilute a reaction, or to change the conditions of a reaction.

As used herein, the term "temperature controlling system" means a device for regulating the temperature of a solid support, particularly the temperature of a liquid present at a target site on a surface of the solid support. A temperature controlling system useful in an open system as disclosed can increase the temperature of a solid support, particularly a target site on the support, or decrease the temperature of the support, as desired. Temperature controlling systems are well known and readily available to those in the art, and are selected, in part, based on the range of temperatures desired, the physical characteristics of the solid support, and its facility of incorporation into a system as disclosed. A temperature controlling system can be, for example, an electrically or electromagnetically regulated heating element or heating/cooling element, such as a Peltier element, or a system that allows contacting the support with, for example, dry ice, liquid nitrogen, or a bath or stream of water maintained at a desired temperature.

As used herein, the term "interface" refers to a system for communicating an amount of liquid to be dispensed to a target site to maintain a predetermined volume. As such, an interface provides a means for controlling an amount of liquid dispensed from a liquid dispensing system. An interface can be in communication, either directly or indirectly, with the target site, with the liquid dispensing system, or with both.

As used herein, the abbreviations for amino acids and protective groups and other such abbreviations are in accord with their common usage and, if appropriate, the IUPAC-IUB Commission on Biochemical Nomenclature [see, (1972) *Biochem.* 11: 1726].

#### Systems

Systems are provided for performing a reaction in an unsealed environment. The disclosed systems and methods provide a means of maintaining a volume of a liquid, for example, a reaction mixture, present in an unsealed environment and, therefore, susceptible to loss of volume by evaporation. In general, the environment into which evaporation can occur is a volume of a gaseous medium, which can be, but need not be, substantially greater than the volume of liquid. A relatively large surface of the liquid can be in direct contact with the environment, and a substantial

amount of a liquid can evaporate into the environment, for example, ten percent or more of the total volume, such that a substantial change in the effective concentration of reactants would occur if the amount of liquid lost due to evaporation is not replaced by a corresponding amount of the liquid dispensed to the volume.

In a system or method as disclosed herein, the liquid generally is present on a surface of a solid support, at a target site, and the environment into which evaporation can occur is air. Many liquids and reactants, including biopolymers, suitable for use in a disclosed system or method are susceptible, for example, to oxidation. Accordingly, an open system as disclosed herein can be placed in a vessel in which the environment can be controlled, for example, the environment can be gaseous medium such as nitrogen, an inert gas such as argon, or other gaseous medium. It should be recognized, therefore, that an unsealed environment may be isolated from the "open air," but nevertheless can be considered an "unsealed environment" for purposes of the present disclosure provided that a liquid, the volume of which is to be maintained, is in contact with a gaseous medium, into which the liquid can evaporate. Further in this regard, various reactions must be performed under conditions of low pressure or high pressure, where the rate of evaporation of a liquid is greater than or less than, respectively, the rate of evaporation of the liquid in open air. An open system as disclosed herein also can be used for performing such reactions, as well as reactions that contain evaporation suppressants or agents that alter the freezing point or boiling point of the liquid, particularly such agents that do not affect a reaction, or for performing reactions in the light, which can be any spectrum of light, or in the dark.

The disclosed systems and methods provide a means to maintain a volume of a liquid at a predetermined volume, where the volume otherwise would decrease below the predetermined volume due to evaporation. An open system can include a solid support having a target site, which can contain a volume of liquid; a liquid dispensing system, which can dispense a liquid to the target site; a temperature controlling system, which can regulate the temperature of the solid support; and an interface, which can indicate an amount of liquid to be dispensed from the liquid dispensing system. In an open system as disclosed herein, the amount of liquid dispensed from a liquid dispensing system can correspond to an amount of liquid lost by evaporation, or can be any predetermined amount of liquid.

The liquid present on a target site can be, for example, a solvent or other component of a reaction mixture. Other components of a reaction mixture can include a substrate, for example, a cell, a biopolymer or an organic or inorganic molecule, and the volume of the reaction can be any desired volume, particularly a submicroliter volume. An open system as disclosed herein can be particularly useful for synthesizing biopolymers such as polynucleotides, polypeptides, polysaccharides and the like, including for synthesizing combinatorial libraries of molecules such as biopolymers, and for performing biological reactions, or chemical reactions using a biopolymer as a substrate, in submicroliter volumes, without concern that evaporation of a liquid from the reaction mixture will undesirably affect the reaction, and, additionally, allows for performing such reactions as single tube reactions.

An open system also can contain a solid support having a target site; a liquid dispensing system, which can dispense a liquid to the target site; a temperature controlling system, which regulates the temperature of the solid support; and means for regulating an amount of liquid dispensed from the

liquid dispensing system. Any means for regulating an amount of liquid dispensed from the liquid dispensing system can be used, including manual manipulation of the liquid dispensing system by an individual monitoring the system, or automatic control of the liquid dispensing system due to an interface between the liquid dispensing system and the temperature controlling device or a temperature sensing device in contact with the solid support.

#### Interface

An interface generally is a component of an automated or semi-automated open system for maintaining a volume of a liquid at a target site. In particular, an interface can be a computerized system that receives input relevant to the volume of a liquid at a target site and, based on that input, provides an instruction to the liquid dispensing system to dispense an amount of liquid that corresponds to an amount of liquid lost from the target site. Thus, an interface for regulating an amount of liquid dispensed by a liquid dispensing system can be, for example, a system for detecting the level of a meniscus, or a computer for receiving input of data from which the volume or level can be calculated. Thus, the interface can include a computer programmed with an appropriate algorithm or software for calculating such level.

Input relevant to the volume of a liquid at a target site can be obtained directly, for example, by detecting a decrease in the level of a meniscus of the liquid or the level of a drop of the liquid below a predetermined point. As disclosed herein, a liquid on a target site can be, for example, in a well or cylinder. In such a case, wherein the liquid is physically surrounded by a barrier, a meniscus forms in the liquid. In addition, a liquid can be placed as a drop on the target site, wherein the liquid is constrained, for example, by the physico-chemical characteristics of the target site. In either case, the level of the liquid can be monitored by detecting a decrease in the level of the meniscus or the drop of liquid.

A decrease in a meniscus below a predetermined point can be detected, for example, by including the liquid in a circuit. In such a system, when the meniscus falls below a predetermined point, which is the point required for the circuit to be complete, a change in the circuit is detected. The interface, upon receiving such input, can indicate that a volume of liquid is to be dispensed to the target site by the liquid dispensing system, until the circuit is reestablished, at which time dispensing of the liquid is terminated. Such a circuit conveniently can be constructed into a microchip using well known methods of photolithography and micro-electronics.

Similarly, where the liquid on the target site has a meniscus or is in the form of a droplet, such input can be obtained by detecting a change in the diffraction, transmission or absorbance of photons as the volume of liquid decreases below a level defined by the positions of an appropriate light source and detector. A system using fiber optics can be useful for monitoring the level of a liquid on a target site and, conveniently, can be included in a detection system, if desired, to monitor the extent of a reaction. As well as the direct methods exemplified above, input relevant to the volume of a liquid at a target site also can be obtained indirectly, for example, using an algorithm that determines the rate of evaporation from the target site based on the temperature of the support containing the target site, the time the temperature has been maintained, and the vapor pressure of the liquid.

A computer with appropriate inputs and outputs, for example, can be used to monitor the temperature of the solid support and, based on various parameters, including, for example, the chemical nature of the liquid, the surface area

of the liquid exposed to the environment, and the time the liquid is maintained at a particular temperature, can estimate the rate of evaporation of a liquid from the target site, and, through an interface, communicate an amount of liquid to be dispensed from the liquid dispensing system to the target site to maintain the liquid at a predetermined volume. For example, a means for regulating an amount of liquid dispensed to a target site can interface with a liquid dispensing system such as a nanoliter dispensing system to compensate for evaporation, thereby maintaining the volume of a liquid at a target site at a predetermined volume (see, e.g., FIG. 1). A computer can directly control the liquid dispensing system to dispense a desired volume, which corresponds to the amount of liquid that evaporates from the target site. The amount of evaporation will depend, in part, on the temperature of the target site, which can be on the surface of a microchip present in a holder that is integrated with, for example, a Peltier element, and a thermistor. The temperature of the support and, therefore, the liquid at a target site, can be any temperature, which can be adjusted based on input from the computer, which is interfaced with the temperature controlling system. Based on the temperature of the microchip, the computer can calculate a rate or amount of evaporation and signal the nanoliter dispensing system accordingly.

A temperature sensing device such as a thermistor produces a signal that indicates the temperature of the support, for example, a microchip support (see FIG. 1). The support temperature signal can be provided to the computer, directly or through the temperature controlling system. Based on the temperature of the support, programming in the computer determines the amount or rate of evaporation and, therefore, a volume of liquid that can be dispensed to the target site to maintain the volume of the liquid at a predetermined level. The computer can provide the temperature controlling system with a signal that indicates the temperature to which the support will be set. Upon receiving the temperature setting signal, the temperature controlling system produces a Peltier element control signal, which directs the Peltier element to adjust the support to the indicated temperature.

An interface need not be directly connected to or control the liquid dispensing system, but can be connected instead to a display, which indicates the amount of liquid needed to be dispensed to maintain the volume of the liquid at a predetermined volume. An individual then can manipulate the liquid dispensing system. In addition, the interface need not be directly connected to the solid support, but can be connected instead to the temperature controlling system and, based on the setting of the temperature controlling system, the chemical and physical nature of the solid support, and the time the temperature is applied to the support, can determine the temperature of the solid support and, therefore, the amount of liquid to be dispensed to the target site. The interface then can display the amount of liquid to be dispensed such that an individual can manipulate the liquid dispensing system, or can transmit the information to the liquid dispensing system, thereby automatically controlling the system.

An open system, as disclosed herein, also can contain means for containing a volume of a liquid; means for dispensing a liquid; means for controlling the temperature of the reaction volume containing means; and means for regulating an amount of liquid dispensed from the liquid dispensing means. A means for regulating an amount of liquid dispensed can include an interface, for example, a computer programmed with software for calculating the appropriate rate or amount. A computer can interface, for example, with

the solid support, thereby monitoring the temperature of the support, and can indicate an amount of liquid to be dispensed to a target site to maintain a predetermined volume of the liquid. The computer can display the amount of liquid to be dispensed and an operator can manipulate the liquid dispensing system such that the amount of liquid is dispensed, or the computer can further interface with the liquid dispensing system, thereby causing the amount of liquid to be dispensed.

#### Solid Supports

A solid support useful in an open system for maintaining a volume of a liquid at a predetermined volume can be constructed of any material having a surface, which can be flat or geometrically altered; for example, to include wells. The solid support is any known to those of skill in the art as matrix for performing synthetic reactions and assays. It can be fabricated from silicon, glass, silicon-coated materials, metal, a composite, a polymeric material such as a plastic, a polymer-grafted material, such as a metal-grafted polymer, or other material as disclosed herein. This material can be further functionalized, as necessary, for example, chemically, to enhance or permit linkage of molecules or other particles, such as cells or cell membranes or viral envelopes or other such biological materials, of interest. The surface of a support can be modified, such as by radiation grafting of a suitable polymer on the surface and derivatization thereof to render it suitable for binding capturing a molecule or particle, such as a cell. The support may also include beads linked thereto (see, copending allowed U.S. application Ser. No. 08/746,036, copending U.S. application Ser. No. 08/933,792, and International application No. PCT/US97/20194, which claims priority to the U.S. applications). It may also include dendrite trees of captured material, or combinations of such additional components. A solid support can have one or more target sites, each of which can contain or retain a volume of a liquid.

By way of example, a solid support can be a flat surface such as a glass fiber filter, a glass surface, a silicon or silicon dioxide surface, a composite surface, or a metal surface, including a steel, gold, silver, aluminum or copper surface, a plastic material, including polyethylene, polypropylene, polyamide or polyvinylidene difluoride, which further can be in the form of multiwell plate or a membrane; can be in the form of a bead (or other geometry) or particle, such as a silica gel, a controlled pore glass, a magnetic or cellulose bead, which can be in a pit of a flat surface such as a wafer, for example, a silicon wafer; or can be a pin, including an array of pins suitable for combinatorial synthesis or analysis (see, e.g., International PCT application No. WO98/20019), comb. microchip. The skilled artisan will recognize that various factors, including the size and shape of the support and the chemical and physical stability of the support to the conditions to which it will be exposed, will be considered in selecting a particular solid support for use in a disclosed system or method.

A solid support contains one or more target sites, which can contain a volume of a liquid. A target site can be, for example, a well, pit, channel, or other depression, with or without rims, on the surface of a solid support; can be a pin, bead or other material, which can be positioned on a surface of a solid support; or can be a physical barrier such as a cylinder, cone or other such barrier positioned on a surface of a solid support.

A target site also can be, for example, a reservoir or reaction chamber, which is attached to a solid support (see, for example, Walters et al., *Anal. Chem.* 70:5172-5176 (1998)). In addition, a target site can be etched, for example,

on a surface of a silicon wafer using a photolithographic method (see, for example, Woolley et al. (*Anal. Chem.* 68:4081-4086 (1996))). Photolithography allows the construction of very small target sites, including wells or towers, and, for example, has been used in combination with wet chemical-etching to construct "picoliter vials" on microchips (Clark et al. *CHEMTECH* 28:20-25 (1998)).

A support also can be a glass or silicon surface containing wells having a very thin base that is transparent to electromagnetic radiation of a desired wavelength, such as laser light, thereby permitting measurement of parameters, such as volume, or an excitation wavelength for fluorescence measurement.

A target site also can be defined by physico-chemical parameters such as hydrophilicity, hydrophobicity, the presence of acidic or basic groups, groups capable of forming a salt bridge, or any surface chemistry that allows a liquid to grow primarily in the z direction. For example, where the liquid to be placed on a target site is water or an aqueous solution, the target site can be defined by a hydrophilic area surrounded by a hydrophobic area on the surface of a solid support, or by a series of rows, alternately having less hydrophobic rows and more hydrophobic rows, whereby the aqueous mixture is constrained to the less hydrophobic rows. With respect to such a target site, the aqueous solution is dispensed, for example, onto the hydrophilic area, and is constrained from spreading from the target site due to the adjacent and surrounding hydrophobic area. Conversely, where the liquid is a nonpolar liquid, it is dispensed onto a hydrophobic region and is constrained in that region due to an adjacent hydrophilic region or a region or that is less hydrophobic than the region to which the liquid is applied.

A solid support can have a single target site, or can contain a number of target sites, for example, 2 sites, 10 sites, 16 sites, 100 sites, 144 sites, 384 sites, 1000 sites, or more, all or some of which can be the same or can be different. Where a solid support contains more than one target site and, therefore, can contain, for example, more than one reaction mixture, the characteristics that define each target site serve not only to constrain a reaction mixture, but also to prevent intermingling of different reaction mixtures or other liquids on the support. In addition, where a solid support contains more than one target site, the target sites can be arranged in any pattern, for example, in a line, a spiral, concentric circles, rows, or an array of rows and columns. Furthermore, the location of each target site of a number of target sites on a support can be defined. The availability of such addressable target sites on a solid support allows multiple reactions to be performed in parallel and is convenient, for example, for performing multiplex reactions, for including control reactions with test reactions such that all are performed under identical conditions, for performing a similar reaction under different conditions, or for performing different reactions.

#### Immobilization of a Reagent to a Solid Support

A substrate or other component of a reaction mixture can be immobilized to a solid support, particularly to a target site on the support, by a covalent interaction or a noncovalent interaction that is stable to the particular conditions of the reaction, as desired. A biopolymer, for example, can be immobilized directly to a solid support, or indirectly, for example, by immobilization to a spacer molecule, which is immobilized to the support. Furthermore, a spacer molecule can be a part of the biopolymer to be immobilized, or can be a separate molecule that directly or indirectly binds the biopolymer, for example, an oligonucleotide including a spacer nucleotide sequence and a sufficiently complemen-

ary probe or primer sequence, which can hybridize to a polynucleotide biopolymer.

Immobilization of a biopolymer can be mediated by a specific binding reaction, for example, by hybridization of a first nucleic acid molecule to a sufficiently complementary second nucleic acid, one of which is immobilized to the support. Similarly, immobilization can be through a first protein to a second protein, one of which is immobilized to the support, for example, an antibody and a polypeptide, which can be expressed on the surface of a cell, having an epitope recognized by the antibody; or an enzyme and its substrate; or any pair of proteins capable of homodimer or heterodimer formation. In addition, immobilization can be between a nucleic acid binding protein and a polynucleotide containing the sequence recognized by the binding protein.

A crosslinking agent also can be used to immobilize a substrate or other component of a reaction mixture to a solid support, through a reversible or irreversible linkage. A useful crosslinking agent can be any agent, including a homobifunctional or hetero-bifunctional agent, that is capable of reacting with a functional group present on a surface of the insoluble support and with a functional group present in the substrate or other component to be immobilized to the support. Useful bifunctional cross-linking agents include N-succinimidyl (4-iodoacetyl) aminobenzoate (SIAB), dimaleimide, dithio-bis-nitrobenzoic acid (DTNB), N-succinimidyl-S-acetyl-thioacetate (SATA), N-succinimidyl-3-(2-pyridylthiol) propionate (SPDP), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-hydrazinonicotimide (HYNIC) (see, also, Wong "Chemistry of Protein Conjugation and Cross-Linking," (CRC Press 1991); Hermanson, "Bioconjugation Techniques" (Academic Press 1995)).

Immobilization of a substrate or other component of a reaction to a solid support can be particularly useful where the crosslink is mediated by a selectively cleavable linker, which can be cleaved under defined conditions. A biopolymer, for example, can be directly linked to a solid support via a reversible or irreversible bond between an appropriate functionality (L') on the biopolymer and an appropriate functionality (L) on the solid support, or on a molecule linked thereto, for example, a spacer molecule. Selectively cleavable linkers include photocleavable linkers and chemically cleavable linkers (see, e.g., International PCT application No. WO98/20019), and enzymatically cleavable linkers such as a polynucleotide sequence containing a particular restriction endonuclease site or a RNase digestion site or a polypeptide sequence containing a particular peptidase site.

Photocleavable linkers, which are cleaved upon exposure to light (Goldmacher et al., *Bioconj. Chem.* 3:104-107 (1992)), include a nitrobenzyl group as a photocleavable protective group for cysteine (Hazum et al., in *Pept. Proc. Eur. Pept. Symp.*, 16th (ed. K. Brunfeldt, 1981), pages 105-110); water soluble photocleavable copolymers, including hydroxypropylmethacrylamide copolymer, glycine copolymer, fluorescein copolymer and methylrhodamine copolymer (Yen et al., *Makromol. Chem.* 190:69-82 (1989)); a cross-linker and reagent that undergoes photolytic degradation upon exposure to near UV light (350 nm; Goldmacher et al., *Bioconj. Chem.* 3:104-107 (1992); nitrobenzyloxy-carbonyl chloride cross-linking agents (Senter et al., *Photochem. Photobiol.* 42:231-237 (1985); and 3-amino-(2-nitrophenyl) propionic acid (Brown et al., *Molecular Diversity*, pages 4-12 (1995); Rothschild et al., *Nucl. Acids Res.* 24:361-66 (1996)). A photocleavable bond such as a charge transfer complex or a labile bond formed between

relatively stable organic radicals can be useful, for example, where the sample is to be examined by mass spectrometry.

A linkage also can be formed with L' being a quaternary ammonium group. Where the sample is to be examined by mass spectrometry, the surface of the solid support also can carry a negative charge, which can repel, for example, a negatively charged nucleic acid backbone and facilitate desorption of a polynucleotide to be detected. Desorption can occur either by the heat created by the laser pulse or, depending on L', by specific absorption of laser energy, which is in resonance with the L' chromophore.

The L-L' chemistry can be a type of disulfide bond, which is chemically cleavable using mercaptoethanol or dithioerythrol; a biotin/streptavidin system; a heterobifunctional derivative of a trityl ether group that can be cleaved under mildly acidic conditions as well as under conditions of mass spectrometry (Köster et al., *Tetrahed. Lett.* 31:7095 (1990)); a levulinyl group cleavable under almost neutral conditions with a hydrazinium/acetate buffer; an arginine-arginine or lysine-lysine bond, which is cleavable by an endopeptidase enzyme such as trypsin; a pyrophosphate bond, which is cleavable by a pyrophosphatase; or a ribonucleotide bond in an oligodeoxynucleotide sequence, which can be cleaved by a ribonuclease or alkali. In addition to acid-labile trityl linkers, acid cleavable linkers include bis-maleimideoxypropyl; adipic acid dihydrazide linkers (Fattom et al., *Infect. Immun.* 60:584-589 (1992)); and acid labile transferrin conjugates that contain a sufficient portion of transferrin to permit entry into the intracellular transferrin cycling pathway (see, for example, Welthöner et al., *J. Biol. Chem.* 266:4309-4314 (1991)).

The L and L' functionalities also can form a charge transfer complex and thereby form a temporary L-L' linkage; a charge-transfer "band" can be determined by UV/vis spectrometry (R. Foster, "Organic Charge Transfer Complexes" (Academic Press 1969)), and the laser energy can be tuned to the corresponding energy of the charge-transfer wavelength so as to effect specific desorption of a sample for a solid support. A reversible L-L' linkage also can be generated by homolytically forming relatively stable radicals, which, under the influence of a laser pulse, for example, during mass spectrometry, desorption and ionization occurs at the radical position. Those skilled in the art will recognize that other organic radicals can be selected and that, in relation to the dissociation energies needed to homolytically cleave the bond between them, a corresponding laser wavelength can be selected (C. Wentrup, "Reactive Molecules" (John Wiley & Sons 1984)).

Thiol-reactive functional groups are particularly useful for immobilizing a biopolymer to a solid support. Thiol-reactive functional groups react with a nucleophilic thiol moiety to produce a covalent bond, for example, a disulfide bond or thioether bond. In general, thiol groups are good nucleophiles, and preferred thiol-reactive functional groups are reactive electrophiles. Thiol-reactive functional groups are known in the art and include, for example, haloacetyls such as iodoacetyl; diazoketones; epoxy ketones;  $\alpha,\beta$ -unsaturated carbonyls such as  $\alpha,\beta$ -enones; and other reactive Michael acceptors, including maleimide, acid halides, benzyl halides, and the like.

A free thiol group present on a polypeptide or incorporated into a polynucleotide can react with a free thiol group such as an iodoacetyl-modified surface (or other thiol-reactive surface functionality) of the support through disulfide bond formation, thereby immobilizing the biopolymer to the support. In addition to being reversible, for example, by exposing the bond to reducing conditions, thiol reactive



linkages also provide the advantage that reaction of a thiol group can be prevented temporarily by blocking with an appropriate protecting group (Greene and Wuts, in "Protective Groups in Organic Synthesis" 2nd ed. (John Wiley & Sons 1991)).

A polynucleotide can be modified at the 3'-terminus or 5'-terminus by reaction with a disulfide-containing modifying reagent, or by enzymatically or non-enzymatically attaching a thiolated primer. A 5'-phosphoramidate functionality can also provide an attachment point for a thiol or disulfide-containing cytosine or deoxycytosine residue. A disulfide-modified nucleic acid can be reduced in a reaction using, for example, tris-(2-carboxyethyl)phosphine (TCEP), at a concentration of about 1 mM to 100 mM, preferably about 10 mM; a pH of about 3 to 6, preferably about pH 4.5; a temperature in the range of 20° C. to 45° C., preferably about 37° C.; and for a time period in the range of about 1 hour to 10 hours, preferably about 5 hours; or using dithiothreitol in a concentration of about 25 mM to 100 mM, depending on whether the reactant is isolated; at a pH in the range of 6 to 10, preferably about pH 8; at a temperature of about 25° C. to 45° C., preferably about 37° C.; and for a time of about 1 hour to 10 hours, preferably about 5 hours. Use of TCE provides an advantage in the low pH at which it is reactive, which effectively protonates thiols, thereby suppressing nucleophilic reactions of thiols and resulting in fewer side reactions than with other disulfide reducing agents used at higher pH ranges.

#### Temperature Controlling System

The temperature of a solid support having a target site is maintained using a temperature controlling system. An open system for maintaining a volume of a liquid at a predetermined volume can include a temperature controlling system, which can heat or cool a solid support, particularly a target site on the support, to a desired temperature. A temperature controlling system is selected, in part, based on the purpose for which the open system is to be used, for example, the reactions to be performed using the open system. As such, a temperature controlling system can be selected that can cool a solid support, for example, to a temperature of 4° C. or less, or 0° C. or less, or -20° C. or less, or -80° C. or less, including, if desired, to about the temperature of dry ice, or liquid nitrogen, or liquid helium; or can heat a solid support, for example, to 25° C. or more, or 37° C. or more, or 45° C. or more, or 65° C. or more, or 90° C. or more, including, if desired, to temperatures greater than 100° C. A temperature controlling system that can heat or cool a solid support within a temperature range of about -20° C. to about 95° C. can be particularly useful as a component of an open system that is to be used for performing biological reactions or reactions involving biological materials, since the reaction volume, including the reactants can be maintained at a low temperature prior to initiating the reaction, then can be adjusted to the appropriate temperature or temperatures for performing the desired reaction.

A thermoelectric module can be particularly useful as a temperature controlling system in an open system as disclosed herein. A thermoelectric module is a solid state device that can be used as a heat pump, utilizing the Peltier effect, to move heat. Depending on the direction that module moves heat, it can be used to heat or cool a solid support. A single thermoelectric module generally can achieve a temperature difference of about 60° C. to 70° C., and several such modules can be used in combination to attain a temperature difference of up to 131° C. Furthermore, by reversing the direction of the current to the module, the direction heat is moved can be reversed. Thus, the thermoelectric module can

be used to reversibly heat and cool a support and, therefore, a reaction mixture such as a PCR reaction located at a target site on the support. Thermoelectric modules are commercially available (Melcor, Trenton N.J.; Americool, Nashua N.H.).

A temperature controlling system such as the Peltier Thermal Cycler (PTC-200 DNA Engine; M. J. Research, Inc., Watertown Mass.) is an example of a temperature controlling system that can be integrated into an open system as disclosed herein. The PTC-200 DNA Engine utilizes a Peltier-Joule heat pump; has a temperature range of -5° C. to 105° C.; provides temperature homogeneity of samples within 30 seconds of reaching 90° C.; accepts a variety of sample supports, including microscope slides and multi-well plates; and conveniently can be interfaced with a computer. An LFI-3526 system, which contains a 22W Peltier element controlled by a programmable thermoelectric temperature controller (Wavelength Electronic, Inc., Bozeman Mont.; see Example 1), is another example of a temperature controlling system useful in an open system as disclosed.

An electronic temperature controlling system also can be incorporated into an open system as disclosed (see Burns et al., *Proc. Natl. Acad. Sci. USA* 93:5556-5561 (1996)). An electronic temperature controlling system can be conveniently constructed, for example, in a microchip using well known methods (see, for example, Woolley et al. *Anal. Chem.* 68:4081-4086 (1996)). Such an electronic temperature controlling system allows thermal cycling using a pulsed width modifier and, therefore, can be useful, for example, for performing PCR reactions (Woolley et al. *Anal. Chem.* 68:4081-4086 (1996); see, also, Ross et al., *Anal. Chem.* 70:2067-2073 (1998); Belgrader et al., *Clin. Chem.* 44:2191-2194 (1998)).

A temperature controlling system also can include a temperature measuring system, which can be used to determine the temperature of a solid support, particularly of a liquid present at a target site on the surface of the support. A temperature measuring system can be, for example, a thermocouple, thermometer, or the like, which can be in contact with a liquid on the support and, therefore, directly determine the temperature of the liquid, or can be in contact with the solid support, thereby providing an indication of the temperature of the liquid. A temperature also can be, for example, an infrared detector, which can monitor the temperature of a liquid at a target site without contacting the support. The use of thermocouples can be particularly convenient because they can be very small in size and can be constructed, for example, into microchips (see Woolley et al. *Anal. Chem.* 68:4081-4086 (1996)). A thermocouple can be in direct contact with a target site, including with a reaction volume at each target site in an array on a solid support, thereby allowing precise monitoring of the temperature of each reaction simultaneously. Input from such thermocouples can be incorporated into an algorithm that allows a calculation of the evaporation rate of liquid from each reaction mixture in an array, and, through the appropriate interface, indicates an amount of liquid to be dispensed to each target site that corresponds to the amount that evaporates from a reaction mixture. Such a means for monitoring the temperature of a number of reaction volumes simultaneously can be particularly useful since different reactions may be being performed at different sites in the array, or because the rate of heating or cooling of different reaction mixtures on a solid support are not identical due, for example, to inhomogeneities in the support or to different concentrations of reagents in a mixture.



The temperature generated by the temperature controlling system and, therefore, the temperature of the solid support, also can be determined based on the particular setting of the temperature controlling system, the physical nature of the solid support, and the time the temperature is applied to the support. Such temperatures can be calculated based on known parameters, or can be determined empirically by heating or cooling a support for incremental periods of time, at incremental temperatures, and measuring the temperature of the support accordingly.

#### Liquid Dispensing/Removing Systems

##### Dispensing

A liquid dispensing system can be an active apparatus, which can be a mechanical, electrical, pressure or pneumatic driven liquid dispensing system, for example, a piezo electric pipette driven by mechanical pressure; or can be a passive apparatus, which contains a reservoir. In addition, a liquid dispensing system can contain a heating element, for example, microresistors, which provides the ability to maintain a liquid in the system at a desired temperature, for example, at or near a reaction temperature.

A liquid dispensing system can include a single fluid transmitting vesicle or multiple vesicles, which can be manipulated independently or together in parallel. A fluid transmitting vesicle can be a solid vesicle, to which the liquid can adsorb and be transferred, or can have a bore, through which the liquid is transferred. Thus, a fluid transmitting vesicle can be a pipet, particularly a micropipet, which contains a chamber for holding or transferring the liquid and an end from which the liquid can be dispensed to a target site; a pin tool, which can have a bore, or can be solid vesicle, which, when dipped into a chamber holding a liquid, adsorbs a volume of the liquid, which then can be transferred to a target site; or a liquid sonicating, vaporizing or ink jet device, which contains a chamber for holding the liquid, and an end from which the liquid is dispensed in droplets, the volume and rate of dispensing of which can be adjusted as desired. A fluid transmitting vesicle can be formed of a metal, composite, glass, silica, or polymeric material, or any other suitable material. A nanoliter liquid dispensing system such as a nanoliter pipet can be particularly useful in a system as disclosed. Nanoliter dispensing systems are provided, for example, in copending allowed U.S. application Ser. No. 08/787,639, U.S. application Ser. No. 08/786,988, and International PCT application No. WO 98/20166, which claims priority to the U.S. applications.

A liquid dispensing system can be part of a liquid handling system, which can contain, in addition to the liquid dispensing system, a chamber for holding a liquid to be dispensed. Such a chamber can be used to directly provide the liquid dispensing system with the appropriate liquid to be dispensed, or can be connected to the liquid dispensing system by a conduit, which mediates transfer of the liquid from the holding chamber to the dispensing system. A conduit can be any suitable conduit, for example, plastic or stainless steel tubing, and can be particularly useful if it can be sterilized without impairing its function. Where it is desirable to dispense a liquid to a target site at a particular temperature, the liquid dispensing system, as discussed above, or a component of a liquid handling system can be maintained at the particular temperature such that the liquid is dispensed at the desired temperature. An advantage of a liquid handling system is that it can contain more than one holding chamber and, therefore, can conveniently allow more than one liquid to be dispensed from a single liquid

dispensing system, for example, from a pin having a bore, without a need to change the position of the liquid dispensing system with respect to the target site. Such a system is particularly convenient where the fluid transmitting vesicle, for example, a pin tool, has an array of fluid transmitting vesicles, which are positioned with respect to a corresponding array of target sites on a solid support.

A liquid dispensing system allows an amount of liquid, preferably a controlled amount, to be dispensed to a target site. The liquid can be dispensed as a continuous stream, or as droplets, which can be dispensed continuously or in a burst mode. The amount of liquid dispensed can be any amount, as desired, including a submicroliter amount or less, and can be dispensed for the purpose of maintaining a liquid at a predetermined volume, or for initiating, terminating or changing the conditions of a reaction at the target site.

A liquid dispensing system can dispense one or more liquids to a single target site, or can dispense one or more liquids serially or in parallel to multiple target sites, which can be in an array. A liquid dispensing system useful for dispensing a predetermined amount of a liquid in parallel can include, for example, an assembly of liquid transmitting systems such as pins, each of which can have a narrow interior chamber suitable for holding a volume of the liquid to be dispensed (see, for example, International PCT application No. WO98/20166). The pins can be fit inside a housing, which can have an interior chamber connected, for example, to a pressure source that regulates the flow of liquid through a pin, thereby allowing controlled dispensing of a predetermined volume of the liquid. Alternatively, the liquid dispensing system can include a jet assembly and a transducer element mounted to a pin, and can dispense an amount of liquid to a target site by spraying the liquid from the pin, or by allowing a drop of the liquid to form on the tip of the pin, where it can be contacted to the target site and dispensed.

A liquid dispensing system can include a single chamber for holding a liquid and, therefore, allow a single liquid to be dispensed, or can contain several chambers, each of which can hold a different liquid and variably can be in connection with the fluid transmitting vesicle. As such, a liquid dispensing system can include a selection element having, for example, a pressure source or a piezoelectric element coupled to a liquid holding chamber and in communication with the fluid dispensing vesicle such that, at a selected pressure condition or a selected voltage, a particular liquid is dispensed at a predetermined amount. Such a selection element conveniently can be interfaced with and controlled by a computer algorithm, which can be monitoring a rate of evaporation of a liquid from a target site, and can allow one or more liquids to be dispensed to a target site, or serially or in parallel to a plurality of target sites. In addition, a liquid dispensing system can dispense a liquid at any desired temperature, particularly the temperature at which a reaction is performed, or a temperature such as about 4° C., which, for example, can suspend a biological reaction. A nanoliter dispensing device, such as the NANO-PLOTTER NP1c (sold by GeSim; Dresden Germany) is an example of a liquid dispensing system that can be incorporated into an open system as disclosed (see Examples 1 and 2). The Nano-Plotter is a modular device that can be combined in a variety of ways depending upon the intended application. It is designed to spot microdroplets arrays onto flat substrates or microwell plates. The device as sold contains from one to eight micropipettes. For use herein, the device can be modified by including heating/cooling elements or heating means to heat the reservoir or the micropi-

petter or other portions thereof, preferably the surface of the target support, to heat or cool the liquid or surface prior to dispensing liquid to avoid a temperature gradient or change upon addition of liquid to a reaction mixture. Other nanoliter dispensing devices can also be used or adapted for use in these systems (see, e.g., copending allowed U.S. application Ser. No. 08/787,639, U.S. application Ser. No. 08/786,988, and International PCT application No. WO 98/20166, which claims priority to the U.S. applications, which describe nanoliter dispensing devices and systems).

The liquid dispensing system can dispense a liquid, which generally is reagent grade or better, or can dispense a solution containing the liquid. For example, in one aspect, the methods as disclosed provide diagnostic assays, the results of which can be analyzed using, for example, mass spectrometry, capillary electrophoresis, a charge coupled device, or a fiber optic system. Where a method such as MALDI-TOF mass spectrometry is used to analyze a component of a reaction, the sample to be analyzed is mixed with an appropriate matrix material (see, for example, U.S. Pat. No. 5,605,798; International PCT application No. WO96/29431; International PCT application No. WO98/20019). As such, a liquid dispensing system can be used to dispense a matrix solution to a target site, prior to subjecting the sample at the target site to mass spectrometry.

#### Liquid Removing

An open system as disclosed herein also can include a device for removing a liquid, which can be a reaction mixture, from a target site and transferring it to another target site or to a chamber for disposal. Such a device provides a convenient means to terminate a reaction, change the reaction conditions, wash a sample, or the like. Accordingly, in an embodiment, the liquid dispensing system also can function to remove a liquid from a target site. The liquid dispensing system, or independent device, can remove a liquid from a target site by contacting the fluid transmitting vesicle to the liquid to be removed and, for example, allowing capillary action to draw the liquid into the vesicle or applying a negative pressure to the vesicle. The removed liquid can be transferred to another location, which can be another target site or a chamber for disposal, and the fluid transmitting vesicle can be washed, if desired, and positioned for further use. A device for removing a liquid from a target site also can be a device that facilitates evaporation of the liquid from the target site, for example, a fan or other device for passing a stream of air or other gas over the liquid.

#### Regulation of Liquid Dispensing System

The liquid dispensing system is regulated so as to dispense a defined amount of a liquid to a target site. The amount of liquid dispensed can correspond to an amount of liquid lost due to evaporation or can be any desired amount of liquid, including a reaction mixture or a solution containing components of a reaction mixture. The liquid dispensing system can be regulated manually or can be regulated semi-automatically or automatically based, for example, on instructions from a computer or other signal transmitting system, which can be interfaced with the temperature controlling system (or a temperature sensing device), with the liquid dispensing system, or with the temperature controlling system (or temperature sensing device) and the liquid dispensing system.

A signal transmitting system can be any system that indicates an amount of liquid to be dispensed. For example, where the amount of liquid to be dispensed corresponds to an amount of liquid lost from a target site due to evaporation,

the signal transmitting system can be any system that provides an indication of the amount of liquid lost. The amount of a liquid lost from a volume in an unsealed environment depends on the vapor pressure of the liquid, which is a function, in part, of the temperature; the surface area of the liquid exposed to the environment; the nature of the environment, including, for example, its relative humidity; and the time during which liquid can be lost. Since these parameters will be known or are determinable for a particular set of conditions, tables can be constructed for predicting an amount of a particular liquid that will be lost in a period of time from a known volume of the liquid applied to a particular target site at a known temperature. Accordingly, for purposes of the present disclosure, such a table is considered a signal transmitting system because an individual, monitoring the temperature and time of a particular reaction, can manipulate the liquid dispensing system to dispense an amount of liquid as indicated by the table.

For semi-automatic or automatic regulation of the liquid dispensing system, the signal transmitting system can be a conventional digital data processing system, for example, an IBM PC compatible computer system, an Apple computer or a UNIX based system, that is suitable for processing data and for executing program instructions that will provide information that can be communicated to the liquid dispensing system. Such a signal transmitting system can be any type of system suitable for processing a program of instructions that will operate the liquid dispensing system, although the system need not necessarily be programmable and can be a single board computer having a firmware memory for storing instructions relevant to regulating the liquid dispensing system.

A signal transmitting system can monitor the temperature of a solid support containing a target site directly, for example, by interfacing with a temperature sensing device, or indirectly, for example, based on the setting of the temperature controlling device and information relating to the chemical and physical nature of the solid support. Alternatively, or in addition, the signal transmitting system can be interfaced with the liquid dispensing system. For example, for semi-automatic operation, the signal transmitting system can be interfaced with either the liquid dispensing system or the temperature controlling system, and the component of the system that is not interfaced with the signal transmitting system can be operated manually by an individual. More conveniently, however, the temperature controlling system and the liquid dispensing system are interfaced with the signal transmitting system, and the entire system is operated automatically.

A signal transmitting system also can interface directly with the target site, including directly with the liquid. For example, a signal transmitting system can include a circuit in a microchip, where the circuit is interrupted by a well in the chip. Upon dispensing a liquid into such a well, the liquid can complete the circuit. In particular, the volume of liquid required to complete the circuit is indicative of the predetermined volume, which is to be maintained. Accordingly, where evaporation of the liquid occurs to the point that the level of the liquid decreases below the level required to maintain the circuit, an indication is provided that a liquid is to be dispensed to the target site. Upon dispensing a sufficient volume of the liquid such that the circuit is reestablished, dispensing of the liquid is terminated.

A signal transmitting system also can include a microbalance, which can detect minute changes in the weight of a support due to evaporation of a liquid from the

surface of the support. In addition, a signal transmitting system can include a light source, which can be of any desired wavelength, and a detector appropriate for the light source. The light can be provided to a target site, for example, using a fiber optic, and the amount of diffraction of the light, or the transmission or absorption of photons can be monitored. A change in the amount of such a parameter can indicate, similarly to the circuit system discussed above, that the level of the liquid has decreased below a predetermined value, or, as discussed below, that an undesirable amount of evaporation has occurred. Such information then can be communicated such that an amount of liquid is dispensed to the target site that corresponds to the amount that has evaporated, thereby maintaining the volume of the liquid at a predetermined level.

A spectrophotometric detector system, for example, can include a laser, which can be a helium-argon laser, a helium-neon laser, an ultraviolet laser, or a laser that emits green or blue light, or can be a light emitting diode (LED), for example, a blue or a green LED. Such a detector system can be particularly useful where the support is a glass or silicon chip, which has wells or the like having a base that allows transmission of the particular wavelength of light. Using such a support, the light can be transmitted from below the chip, through the sample, and can be detected by a detector placed above the well. The light transmitting system and detector can be a single source, or can be arranged in an array that corresponds to positions of the target sites on the support. Furthermore, such a spectrophotometric system can be separate from the open system for maintaining a volume of a liquid in an unsealed environment, and the support can be repositioned to the detector system when desired. Preferably, however, the spectrophotometric system is integrated into the open system, thus allowing online monitoring of a reaction. In such an integrated system, a temperature controlling system such as a Peltier element is constructed with holes at positions corresponding to the target sites, particularly to the position at which the light source transmits the light to the support.

The use of a spectrophotometric system can allow monitoring of a reaction, for example, where the reactants are labeled with an appropriate fluorescent, luminescent or chemiluminescent moiety, for example, a reaction performed using the TaqMan™ assay (see Example 1). The light transmitting system and detector are selected based on the particular wavelength of light desired. Such a spectrophotometric system also is useful for monitoring the volume of a liquid at a target site, as disclosed above, for example, by detecting a change in the diffraction, transmission or absorbance of photons that reach the detector. Depending on the particular liquid at the target site, including the desired predetermined volume to be maintained, the reactants, when present, in the liquid, and the like, tables can be constructed that indicate, for example, the amount of light transmitted to a detector that signals an undesirable amount of evaporation of the liquid, such that a liquid dispensing system dispenses an amount of liquid that corresponds to the amount that evaporated.

#### Detection Systems

A reaction mixture, including a component added to the mixture, for example, a substrate, or an intermediate or product produced by the reaction, can be monitored using any detection system appropriate for the material being examined. The detection system is selected based in the particular material to be detected, and can be matched with a particular label where the material to be detected based on

identifying the presence of a label attached to the material. Since the disclosed systems and methods are particularly useful for performing reactions in small volumes, particularly submicroliter volumes, the material to be detected generally is present in only a very small amount. Accordingly, the detection system is selected, in part, on its sensitivity for detecting the material.

A detection system can be a photometric or spectrophotometric system, which can detect ultraviolet, visible or infrared light, including fluorescence or chemiluminescence; a radiation detection system; a spectroscopic system such as nuclear magnetic resonance spectroscopy, mass spectrometry or surface enhanced Raman spectroscopy; a charge coupled device; a system such as gel or capillary electrophoresis or gel exclusion chromatography; or other detection system known in the art, or combinations thereof.

A mass spectrometry detection system can be useful in an open system as disclosed because it can detect the presence of very small amounts of a material, for example, a biopolymer, and, at the same time provides an indication of the identity of the detected material. In addition, mass spectrometry does not require labelling a material to be detected, although the materials can be "labeled," for example, by incorporating mass differentiating functional groups into the materials where a multiplex reaction is to be performed. Mass spectrometry also is useful because the systems and methods disclosed herein can utilize a solid support such as a microchip, which can be introduced conveniently into the mass spectrometer.

A useful mass spectrometry detection system can be any of various formats, including ionization (I) techniques such as matrix assisted laser desorption (MALDI), continuous or pulsed electrospray (ESI), ionspray, thermospray, or massive cluster impact (MCI). Such ion sources can be matched conveniently with a detection format, including linear or reflectron time-of-flight (TOF), single or multiple quadrupole, single or multiple magnetic sector, Fourier transform ion cyclotron resonance (FTICR), ion trap, and combinations thereof to yield a hybrid detector, for example, ion-trap/time-of-flight. For ionization, numerous matrix/wavelength combinations (MALDI) or solvent combinations (ESI) can be employed. MALDI-TOF mass spectrometry, including delayed extraction MALDI-TOF mass spectrometry is particularly useful as a detection system (see, for example, International PCT application No. WO98/20019; see, also, Whittall et al., *Anal. Chem.* 70:5344-5347 (1998), describing the use of MALDI-TOF mass spectrometry for the analysis of proteins isolated from single cells).

A charge coupled device (CCD) camera can be useful for detecting and imaging fluorescent, chemiluminescent or radionuclide labeled materials. Such a detection system has been adapted to analysis of reactions on microchips, and can allow the detection of thousands of binding reactions performed on a microchip (see Eggers and Ehrlich, *Hematol. Pathol.* 9:1-15 (1995); see, also, Eggers et al., *Biotechniques* 17:516-525 (1994). Using a CCD detecting system, biopolymers, which can bind an appropriately labeled material, or reagents that can bind an appropriately labeled material such as a fluorescently labeled biopolymer, are immobilized directly on the pixels of a CCD, and, following a reaction as desired, bound labeled materials are detected at the specific pixel location. The signal obtained from a CCD can be displayed, if desired, and can allow a quantitative determination, for example, of binding events. Furthermore, since the signal obtained using a CCD detector is received in real time, the signal can be used as an indication of the

extent of a reaction, and can be interfaced with the liquid dispensing system to cause an amount of a liquid to be dispensed, for example, to terminate the reaction, or to change the conditions of the reaction such that a second reaction can be performed at the particular target site.

Where a substrate, or a product of a reaction to be detected, is labeled using, for example, a luminescent, fluorescent or chemiluminescent label, a fiber optic system can be used as a detection system (see, for example, Clark et al. *CHEMTECH* 28:20-25 (1998)). Fiber optics are particularly useful because their small size permits the monitoring of individual target sites, for example, in an array of target sites on a microchip. In addition, a fiber optic system can provide the additional function of monitoring the level of a liquid, as disclosed above. Other detection systems including laser scanners (Cheung et al., *Nature Genet.* 21:1519 (1999)), capillary electrophoresis (Hadd et al., *Anal. Chem.* 69:3407-3412 (1997)), and epifluorescence microscopy (Fodor et al., *Science* 251:767773 (1991)) have been adapted to microchip devices and glass slides and can be used in an open system as disclosed herein.

#### Methods

Methods for maintaining a volume of a liquid in an unsealed environment also are provided. Such a method can be performed in an open system, for example, by determining the temperature of a solid support, having a target site, which can contain the volume of liquid; and, based on the temperature, dispensing at the target site an amount of liquid required to maintain the volume of the liquid. The amount of liquid to be dispensed can be determined, for example, using a computer algorithm that can calculate, based on various parameters, including the temperature of the support, the chemical nature of the liquid, and the volume to be maintained, the amount of liquid that evaporates from the site, such that the amount of liquid dispensed corresponds to the amount of liquid that evaporates. Alternatively, the volume can be monitored, for example, by a microscopic examination, using an appropriate optical system, or by a video imaging technique, such that, as the volume of a liquid at a target site decreases, liquid can be dispensed to maintain the volume within acceptable parameters. In another embodiment, the volume can be monitored by tracking the meniscus of the liquid and determining when the meniscus decreases below a defined point. Such monitoring can be performed by detecting a change in a circuit due to a decrease in the amount of liquid below a level required to maintain the circuit, or by a change in the quality of light being transmitted into the liquid.

Methods for performing a reaction in an unsealed environment also are provided. Such a method can be performed, for example, by determining the temperature of a solid support, which includes a target site containing a volume of the reaction mixture; and dispensing into the reaction mixture an amount of liquid required to maintain the volume. Such a method is particularly useful where the reaction mixture has a volume of a few microliters or less, generally a volume of about 20 microliters or less, and particularly about 500 nanoliters or less. The disclosed methods also are useful for performing submicroliter reactions at temperatures where the vapor pressure of a liquid in the reaction mixture is undesirably high, for example, about 2.5 kilopascals (kPa) or greater, particularly about 5 kPa or greater, or about 10 kPa or greater, such that evaporation of the liquid can substantially change the volume of the reaction mixture and adversely affect the reaction.

The disclosed methods are useful, for example, for performing a reaction in an aqueous environment at a tempera-

ture greater than about 22° C. (room temperature; RT; 72° F.), particularly at a temperature about 37° C. or greater, where the vapor pressure for water is 2.6447 kPa at 22° C., 6.2795 kPa at 37° C., and 84.529 kPa at 95° C. ("Handbook of Chemistry and Physics" 75th ed. (CRC Press, Inc., 1994); see pages 6-15 to 6-17; see, also, 6-77 to 6-108; and 15-43 to 15-49). As such, the disclosed methods can be used, for example, to perform various chemical, physical and biological reactions such as synthesis of a combinatorial library, a mammalian cell transfection, or a polymerase chain reaction.

A method for maintaining a volume, particularly a sub-microliter volume, of a reaction mixture on a solid support in an unsealed environment can be performed by determining the rate of evaporation of a liquid from the reaction mixture; and dispensing into the reaction mixture an amount of liquid that corresponds to the amount of liquid that evaporates, thereby maintaining the volume of the reaction mixture.

Such a method can be useful, for example, where the reaction mixture contains a biopolymer, which can be a substrate or a product of the reaction. Following a reaction using a method as disclosed herein, the biopolymer or products of the biopolymer can be detected, either directly or indirectly. As such, the disclosed methods are useful for determining the sequence of a biopolymer, for synthesizing a biopolymer from monomeric subunits, and for detecting the presence of a particular biopolymer, for example, in a biological sample. Various methods for detecting a biopolymer are exemplified herein and other methods, which are selected based, in part, on the particular type of biopolymer, are well known to those in the art.

A method as disclosed herein can be useful for essentially any type of reaction, including, for example, where the substrate is a biopolymer, a biological reaction such as an enzyme-mediated polymerization, ligation, cloning, or a degradation reaction; a physical reaction such as a nucleic acid hybridization, the binding of a nucleic acid regulatory element by a particular nucleic acid binding polypeptide, or homodimerization or heterodimerization of polypeptides; or a chemical reaction such as a chemical labeling reaction, chemical synthesis of the biopolymer; or a chemical cleavage of the biopolymer, for example, cyanogen bromide cleavage of a polypeptide at a methionine residue or dimethylsulfate cleavage of a carbohydrate end group. The reaction also can be a chemical synthesis reaction, for example, synthesis of a combinatorial library of small molecules or of biopolymers; or can be a hydrolysis reaction such as a polysaccharide hydrolysis reaction.

A method as disclosed herein also is useful for a reaction involving a living cell. For example, the reaction can be a method of introducing a recombinant nucleic acid molecule, which can be contained in a vector, into a host cell in order to produce copies of the recombinant nucleic acid molecule or to express a polypeptide encoded thereby. Such a polypeptide can be isolated, if desired, or can be expressed for the purpose of providing an advantage to the cell expressing the polypeptide. The reaction also can involve contacting a cell with a physical, chemical or biological agent in order to identify a change in gene expression in the cell, for example, by imposing a heat shock on the cells, or by contacting the cells with a putative medicament. An open system as disclosed herein is useful for such reactions because it allows precise control of the reaction conditions, particularly the ability to maintain the reaction at a predetermined volume. Furthermore, because the reactions can be performed in very small volumes, studies involving only one

or a few cells can be performed (see, for example, Clark et al. *CHEMTECH* 28:20-25 (1998)).

#### Sequencing Reactions

The methods disclosed herein can be used for a biopolymer sequencing reaction. For example, the biopolymer can be a polynucleotide, which can be sequenced using the Maxam-Gilbert chemical cleavage method, or an enzymatic reaction such as the Sanger-Coulson chain termination method or an exonuclease cleavage method. A biopolymer sequencing reaction such as the Maxam-Gilbert method or Sanger-Coulson method conveniently can be performed, for example, on a microchip, in which a number of reactions, including the four (or five) base specific reaction, can be performed in parallel on one or more polynucleotides, or a single base reaction can be performed on a number of different polynucleotide sequences. Such methods of polynucleotide sequencing result in the production of nested fragments of the polynucleotide, which can be detected using various methods, particularly mass spectrometry, including matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry or capillary electrophoresis.

In a chain termination sequencing reaction, for example, each reaction mixture is an aqueous solution, containing a polymerase, four nucleoside triphosphates, and a chain terminating nucleoside triphosphate; the water in the reaction mixture is susceptible to evaporation during the reaction, which generally is performed at a temperature of about 37° C. Particularly where the reaction is performed in a submicroliter volume on a solid support in an unsealed environment, the water can evaporate almost completely in a short period of time and, even where the reaction mixture does not evaporate to dryness, the loss of water volume can substantially alter the kinetics of the polymerase reaction, including the fidelity of nucleotide incorporation, and produce spurious results. A method as disclosed herein avoids such a problem by dispensing into the reaction mixture an amount of water that corresponds to the amount of water that evaporates during the reaction.

In the Maxam-Gilbert sequencing method, four or five separate reactions can be performed, each of which is performed under different conditions, with different liquids, including water, dimethyl sulfate, piperidine, and hydrazine (see Sambrook et al., "Molecular Cloning: A laboratory manual" 2nd ed. (Cold Spring Harbor Laboratory Press 1989), pages 13.11-13.13). For example, a sugar-phosphate cleavage reaction of the modified bases is performed using 1 M piperidine in water at 90° C. (vapor pressure of piperidine=84.1 kPa at 100° C.; and of water=101 kPa at 100° C.). As such, a method as disclosed herein, can be used to monitor the evaporation of piperidine and of water during the sugar-phosphate cleavage reaction and can allow an amount of each liquid to be dispensed to the target site during the reaction. The relative amount of each such liquid to be dispensed can be calculated, for example, based on the concentration of each liquid in the reaction and the vapor pressures of the liquids.

A polynucleotide also can be sequenced by an exonuclease reaction using a method as disclosed herein. For example, a multiplex exonuclease sequencing reaction can be performed on polynucleotides as disclosed in U.S. Pat. No. 5,622,824 and U.S. Pat. No. 5,851,765, wherein mass differentiated nucleic acid molecules containing mass modified nucleotides are prepared, immobilized to a target site, and contacted with an exonuclease. A number of exonuclease sequencing reactions can be run in parallel, for example, on a microchip, and the concentration of

exonuclease, pH, and time of incubation can be varied in the different reaction mixtures to produce a desired range of degradation products. Time of incubation can be adjusted, for example, by maintaining the temperature of each target site at 4° C., then at predetermined times, adjusting the temperature of one or more target site to 37° C. The volume of each reaction mixture is maintained at a predetermined volume throughout the procedure. At the appropriate time, all of the reactions are terminated, for example, by removing the liquid and dissolved reagents from the target sites. The immobilized, exonuclease degraded mass differentiated nucleic acid molecules remain immobilized to the chip and are available for detection, for example, by mass spectrometry.

A polypeptide also can be sequenced using a chemical degradation method, for example, the Edman degradation method, which utilizes phenylisothiocyanate to sequentially cleave amino acids from the amino terminus of a polypeptide, or an enzymatic degradation reaction using an exopeptidase such as a carboxypeptidase, which sequentially cleaves amino acids from the carboxy terminus of a polypeptide, or an aminopeptidase, which sequentially cleaves amino acids from the amino terminus of a polypeptide.

The sequentially released amino acids or nested fragments of the polypeptide can be detected. Nested fragments of a polypeptide can be produced conveniently by performing a number of reactions in parallel on a microchip, where the polypeptides are reversibly immobilized to the solid support using, for example, photocleavable linkers, chemically cleavable linkers, or the like. After the reactions are complete and the immobilized fragments have been washed, they can be detected in situ, or can be released from the support for detection.

The methods as disclosed herein also can be useful for reactions in which a biopolymer is cleaved into smaller, but not necessarily monomeric, fragments. For example, a polynucleotide can be cleaved, partially or completely, using a restriction endonuclease or base specific endonuclease such as various RNA endonucleases. Similarly, a polypeptide can be cleaved into fragments using, for example, cyanogen bromide, which cleaves at methionine residues, or any of various endopeptidases such as trypsin and chymotrypsin. The fragments produced then can be detected to facilitate determining the order of the fragments within the larger polynucleotide or polypeptide. The methods disclosed herein also are applicable where the biopolymer is a carbohydrate, glycoprotein, proteoglycan or lipid, which is to be sequenced.

#### Synthesis Reactions

The disclosed methods for maintaining a volume, particularly a submicroliter volume, of a reaction mixture also are useful for a biopolymer synthesis reaction. For example, the biopolymer can be a polynucleotide, and the synthesis reaction can be a chemical synthesis reaction or an enzymatic synthesis reaction using a polymerase (see, for example, S. M. Hecht, ed. "Bioorganic Chemistry: Nucleic acids" (Oxford Univ. Press 1996)). Chemical synthesis of a polynucleotide can be performed using any of various methods, including the phosphotriester, phosphoramidate and H-phosphonate method (Hecht, ed. "Bioorganic Chemistry: Nucleic acids" Oxford Univ. Press 1996, pages 36-74), and utilizes various organic solvents, including, for example, acetonitrile, which has a vapor pressure of about 11.8 kPa at 25° C. and, therefore, is more susceptible to evaporation than water. As such, the disclosed methods are particularly useful for chemical polynucleotide synthesis

reactions, since the volume of the reaction mixtures can be maintained at a predetermined volume throughout the reaction period.

An enzymatic synthesis reaction, in comparison, is performed in an aqueous solution. The biopolymer can be a ribonucleic acid, and the polymerase can be an RNA dependent RNA polymerase or an RNA dependent DNA polymerase. Where the polymerase is an RNA dependent DNA polymerase, the enzymatic synthesis reaction also can include a DNA dependent DNA polymerase, for example, a reverse transcription polymerase chain reaction (RT-PCR).

A polynucleotide can be synthesized, for example, by PCR. In addition to the substrate polynucleotide and a polymerase, which can be a DNA polymerase or RNA polymerase, other components of a PCR reaction include nucleoside triphosphates, which can be deoxyribonucleotides, ribonucleotides or analogs thereof, and a set of primers, including a forward primer and a reverse primer. Nested PCR reactions also can be performed, in which case a second set of primers, which specifically hybridize to the first amplification product, are a component of the reaction. A primer can be any oligonucleotide, including an oligonucleotide containing oligonucleotide mimetics, such as PNA (protein nucleic acid formed by conjugating bases to an amino acid backbone, which render the base sequence less susceptible to enzymatic degradation; see, e.g., Nielsen et al. (1991) *Science* 254:1497), portion(s), provided that the nucleotide at the 3' end of such a primer is linked to the oligonucleotide by a phosphodiester bond, or the like, such that extension of the primer from the 3' end can occur. The disclosed methods of maintaining a reaction mixture in an unsealed environment at a predetermined volume are particularly valuable for performing a PCR reaction, since PCR utilizes a number of different steps, performed at different temperatures, including temperatures as high as 95° C. Thus, the disclosed methods provide a means to monitor the reaction volume at different times during the PCR reaction and dispense liquid, generally water, to the target site in order to maintain the reaction at a predetermined volume.

The disclosed methods for synthesizing a biopolymer in a submicroliter reaction also can be used to synthesize a polypeptide. The disclosed methods also can be used to synthesize a carbohydrate, a glycoprotein, a proteoglycan or a lipid in a submicroliter reaction mixture.

#### Diagnostics

Genetic factors may contribute to virtually every human disease, conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental influences. Much of current biomedical research, in the public and private sectors, is based upon the expectation that understanding the genetic contribution to disease will revolutionize diagnosis, treatment, and prevention. Analysis of DNA sequence variation is becoming an increasingly important source of information for identifying the genes involved in disease and in normal biological processes such as development, aging and reproduction.

Genomic research has identified several types of DNA sequence variations, including insertions and deletions, differences in the copy number of repeated sequences, and single base pair differences such as single base pair deletions, termed single nucleotide polymorphisms (SNPs). SNPs occur with a frequency of about 1% (or about 1 million SNPs) in the human genome and serve as markers of regions in the human genome. While biological processes and diseases are caused or influenced by complex interactions among multiple genes and environmental factors,

many alleles associated with health problems may have low penetrance, meaning that only a few of the individuals carrying them will develop disease. SNPs better identify regions important for mapping and discovering the genes associated with common diseases. In addition to their frequency, SNPs are attractive candidates as genetic markers due to their stability, generally having much lower mutation rates, and the amenability of automating the analysis of such sequences, thereby allowing large scale genetic analysis.

The screening and scoring of the million or so SNPs and the genetic loci predicted make up the human genome largely is dependent on the scientific community's ability to reduce the cost of such analysis. Over the past few years, scientists have begun to develop methods such as multiplexing, which allow the analysis of more than one genetic locus per sample. Further development of such methods has led to the use of nanotechnology, which has miniaturized sample preparation and biochemical reactions, allowing significant cost savings and movement of DNA analysis toward automation. The open systems and methods disclosed herein provide a substantial step forward in adapting nanotechnology to the analysis of biopolymers, including in situ biopolymer synthesis and sequencing, and diagnostic assays such as oligonucleotide based primer extension reactions and PCR.

#### Methods of Detecting the Presence of a Biopolymer

The disclosed methods of performing a reaction in a submicroliter volume in an unsealed environment are useful for detecting the presence of the biopolymer, which can be in a biological sample, because of the ability to perform such assays with only a small amount of sample. As such, the disclosed methods are particularly useful for performing clinical diagnostic assays.

The biopolymer can be, for example, a polynucleotide, which is immobilized to the solid support and detected by identifying a detector oligonucleotide that hybridizes to the biopolymer. The detector oligonucleotide can be a peptide nucleic acid. The method can be performed with a plurality of reaction mixtures, wherein one or more of the plurality of reaction mixtures contains a biopolymer, which can be immobilized to the solid support. In such a method, the solid support can be a microchip, and the plurality of reaction mixtures is present in an array on the microchip.

Any component of the reaction mixture can be detected, as desired, including, for example, the biopolymer, which detected directly or indirectly. For example, where the biopolymer is a polynucleotide, it can be detected by identifying an amplification product produced from the polynucleotide, or by identifying a reagent such as an oligonucleotide that binds specifically to the biopolymer. The oligonucleotide can include a PNA portion, and can be an oligonucleotide primer that has been extended due to the activity of a polymerase. Where the biopolymer is a polypeptide, the reagent can be a second polypeptide that binds specifically to the first polypeptide, for example, an antibody.

The disclosed methods of performing a reaction in a submicroliter volume in an unsealed environment also can be used to examine a polynucleotide using a primer extension reaction. The primer extension reaction can be competitive oligonucleotide single base extension; primer oligo base extension (PROBE); loop-PROBE; or telomeric repeat amplification protocol, and the primer can be any oligonucleotide, including, for example, an oligonucleotide containing a peptide nucleic acid portion and having a 3' terminus that is a substrate for a polymerization reaction (see, e.g., International PCT application No. WO98/2001 9).

Such methods provide diagnostic assays, including assays for detecting the presence of, or predisposition to a disease or condition. Such a disease or condition can be a genetic disease, for example, Huntington's disease, prostate cancer, Fragile X syndrome type A, myotonic dystrophy type I, Kennedy's disease, Machado-Joseph disease, dentatorubral and pallidolusian atrophy, and spino bulbar muscular atrophy; or the condition can be aging, which can be identified by examining the number of nucleotide repeats in telomere nucleic acid from a subject. The disease or condition also can be associated with a gene such as genes encoding BRCA1, BRCA2, APC; a gene encoding dystrophin,  $\beta$ -globin, Factor IX, Factor VIIc, ornithine-d-amino-transferase, hypoxanthine guanine phosphoribosyl transferase, or the cystic fibrosis transmembrane receptor (CFTR); or a proto-oncogene.

The methods as disclosed herein also are useful for detecting single nucleotide polymorphisms (SNPs), which occur with a frequency of about 1% (or about 1 million SNPs) in the human genome and serve as markers of regions in the human genome. SNPs can identify regions important for mapping and discovering the genes associated with common diseases, and are attractive candidates for analysis because of their frequency in the human genome and because of their relatively low mutation rates. As such, analysis of SNPs is amenable to automation.

A method as disclosed herein can be used to genotype a subject by determining the identity of one or more allelic variants of one or more polymorphic regions in one or more genes in the subject. For example, the one or more genes can be associated with graft rejection and the process can be used to determine compatibility between a donor and a recipient of a graft. Such genes can be MHC genes, for example. Genotyping a subject using a process as provided herein can be used for forensic or identity testing purposes and the polymorphic regions can be present in mitochondrial genes or can be short tandem repeats.

A disclosed method also can be used to determine whether a subject is infected with an infectious organism such as a virus, bacterium, fungus or protist. A process for determining the isotype of an infectious organism also is provided. Thus, depending on the sequence to be detected, the methods disclosed herein are useful, for example, to diagnose a genetic disease or chromosomal abnormality; a predisposition to or an early indication of a gene influenced disease or condition, for example, obesity, atherosclerosis, diabetes or cancer; or an infection by a pathogenic organism, for example, a virus, bacterium, parasite or fungus; or to provide information relating to identity, heredity or compatibility using, for example, mini-satellite or micro-satellite sequences or HLA phenotyping.

#### Libraries

The disclosed methods for performing a reaction in an unsealed environment are useful for producing libraries containing diverse populations of molecules, including chemical or biological molecules such as simple or complex organic molecules, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, polynucleotides, and the like. Libraries containing such molecules and methods of making libraries, such as combinatorial libraries, are known in the art (see, for example, Huse, U.S. Pat. No. 5,264,563; Gallop et al., *J. Med. Chem.* 37:1233-1251 (1994); Gordon et al., *J. Med. Chem.* 37:1385-1401 (1994); Blondelle et al., *Trends Anal. Chem.* 14:83-92 (1995); Eichler and Houghten, *Molec. Med. Today* 1:174-180 (1995); York et al., *Science* 274:1520-1522 (1996); Gold et al., *Proc. Natl. Acad. Sci., USA* 94:59-64 (1997); Gold, U.S. Pat. No. 5,270,163, issued Dec. 14, 1993).

Libraries, such as combinatorial libraries, can contain as many as  $10^{14}$  to  $10^{15}$  different molecules, and typically contain on the order of  $10^3$ - $10^6$ . The diverse molecules in a combinatorial library can be based, for example, on a known molecule, such as known pharmacophore scaffold, which is being diversified to find a new, but similar molecule having more desirable characteristics such as better solubility or the ability to be administered orally or bioactivity. Of course, the diverse molecules also can be randomly designed molecules, which can be screened for a desirable characteristics.

The methods herein allow libraries of diverse molecules to be produced in an open system and, if desired, using a single tube format. In addition, the diverse molecules produced can be screened in situ, for example, where the library is a library of diverse antibodies, they can be screened using the appropriate antigen and a suitable binding assay; or where the library is a library of diverse drugs, which are based on a drug that can inhibit a protein-protein interaction involved, for example, in a metabolic pathway associated with a disease, the diverse drugs can be screened by contacting a drug with the proteins and monitoring the association of the proteins. A screening assay provides a simple means for identifying those agents in the library that have a desirable property. Thus, a screening assay can be performed following preparation of a combinatorial library, and the entire process can be automated using an open system as disclosed, thereby allowing for high through-put screening assays.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

#### EXAMPLE 1

##### Unsealed Nanoliter PCR Monitored by Fluorescence Energy Transfer Assay in a Single Well Procedure

This example demonstrates that a PCR amplification performed in an open system can be detected online by increasing fluorescence using a fluorescence energy transfer assay, the TaqMan™ assay (*Nucleic Acids Res.* 25:1999-2004 (1997)).

The TaqMan™ fluorescent assay uses an oligonucleotide probe complementary to an internal segment of the target DNA to be amplified. The probe is labeled with two fluorescent moieties. As a result of the overlap between the emission and excitation spectra of the two fluorescent moieties, one moiety quenches the emission of the other moiety. The presence of this probe during PCR allows the amplification process to be monitored. The probe hybridizes to the target DNA during the PCR process and becomes susceptible to degradation by the 5' nuclease activity of Taq polymerase, which is specific for DNA hybridized to the template. As a result of the nucleolytic degradation, the two fluorescent labels are no longer in proximity, thereby reducing the quenching and increasing the intensity of the emitted light. As a result, measurement of fluorescence during amplification permits real-time monitoring of the PCR yield.

A TaqMan™ kit (Applied Biosystems, Foster City Calif.) contains the following components: human genomic DNA at a concentration of 10 ng/ $\mu$ l, forward and reverse primers specific for the human  $\beta$ -actin gene: forward primer 5'-TCACCCACACTGTGCCCATCTACGA-3' (SEQ ID No. 1); and reverse primer (5'-CAGCGGAACCGCTCATTGCCAATGG-3' (SEQ ID No. 2); a dual fluorophore-labeled probe: 5'-(6-carboxyfluorescein)-ATGCCC-(6-carboxytetramethylrhodamine)-CCCCCATGCCATCCTGCGT-3' (SEQ ID No. 3)



complementary to the  $\beta$ -actin specific PCR product. The reaction mixture contains 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 1 mg/ml bovine serum albumin (BSA), 3.5 mM  $MgCl_2$ , 200  $\mu$ M of each dNTP, 300  $\mu$ M of the forward and reverse amplification primers, 200  $\mu$ M of the dual fluorophore-labeled probe, 0.5 Units Taq polymerase, 0.1 Units anti-Taq antibody and 5 ng of template DNA in  $\mu$ l total volume. PCR is performed using the following cycling conditions: 40 cycles of 94° C. for 10 seconds, 54° C. for 5 seconds, and 72° C. for 15 seconds.

PCR was performed on a modified 2 channel NANO-PLOTTER pipetting device type NP1c (GeSim, Dresden Germany). This fluid dispenser device has an xyz table to move and dispense liquids from a piezo electric pipette. The pipettes are connected to pump system (diluters) to fill the pipettes with liquid in the nanoliter scale from a microliter plate deposited on the z-table of the pipetting device. The other end of the diluters are connected to a reservoir which contains system liquid, for example, 50 ml ultrapure water. Valve settings in the diluters allows to bypass system liquids to the piezo electric pipettes.

The NANO-PLOTTER (GeSim, Germany), discussed above, is modified so that the target sites includes a 22W Peltier heating/cooling element, or other such element, which is controlled by a programmable thermoelectric temperature controller LFI-3526 (Wavelength Electronic, Inc., Bozeman Mont.). Modification with reference to the NANO-PLOTTER refers to coupling the device with a heating element to heat the liquid before it is dispensed onto the chip support. This can be effected by heating of the microtiter dish or heating the source of the water, such as the reservoir. Temperature is measured using a PT 500 type F3132 (Newport Electronics, Deckenpfronn Germany). System liquid is heated to target temperature directly in the pipette to keep the temperature gradient between reaction liquid on the target and subsequently added system liquid zero.

The reaction is deposited on two different positions on a microchip support (which will be sold under the trademark Spectrochip™ by Sequenom, Inc., San Diego Calif.), which has a hydrophobic surface with hydrophilic target sites for retaining aqueous reaction mixtures. The chip support contemplated in this example, includes two modified hydrophobic positions that allow the reaction liquid to grow only in the z-direction. One position on the chip is used to monitor the reaction using a fiber optic set very close to the reaction position. The fiber optic is connected to a photomultiplier to convert the fluorescence signal to an electric signal. The bandpass filter is placed between the fiber optic and the photomultiplier to cut-off the exciting wavelength. Fluorescence is excited with a 15 mW argon laser and detected through the recommended bandpass filter (Applied Biosystems).

The second position on the chip (dummy position) is used to monitor the liquid loss due to evaporation. An inter digital array is set very close to the dummy position in order to monitor drop size by capacity measurements. The reaction mix and the dummy drop are kept constant during each PCR cycle by adjusting the dispensing frequency of the system liquid. Since the capacity can be changed in both directions, when the drop volume increased or decreased, the frequency of added reservoir liquid is related to the cycle program, so that the slope of frequency is negative when the cycle temperature is decreased and is positive when the cycle temperature is increased. The whole system can be covered and darkened to reduce background effect while obtaining the fluorescence signal.

Five nl (25 drops) of reaction mix and dummy liquid are transferred from a microtiter plate onto a cooled (5° C.) 2-position silicon Spectrochip™ microchip. This cooled trap is used to determine the initial drop size, without evaporation. Once the initial drop size is determined the PCR program is started. During PCR, the pipettes replace evaporated water from the system liquid based on the measured capacity and fluorescence signal, as obtained online. Liquid loss also can be monitored by laser scan microscopy, where the whole dummy drop is irradiated by a He-Ne laser (632 nm) and monitored (Fraunhofer Inst., Erlangen Germany). Each deviation of drop size is recognized and liquid is dispensed accordingly.

For a 97 tube procedure, including 96 reactions and one dummy reaction, 97 very small laser diodes (2x2 mm) are placed under the target sites to excite fluorescence in the reaction volume from behind. An advantage of this system is that each of the 97 reactions can be monitored at the same time without scanning the argon laser over the whole chip. The reaction volume is dispensed onto a glass pad, and the laser diode is mounted under the Peltier element to provide the diode with its working temperature, because the storage temperature of a laser diode is lower than the temperature used in a reaction such as PCR and, therefore, cannot be placed between the target and the peltier element. Thus, the Peltier element is constructed with holes to allow passage of the focused laser beam. A 97 diode array also can be placed at a different location.

The same experiment, in a 97 tube or 385 tube format, can be performed using two different blocks, containing active and passive piezo electric pipettes. The active block contains 1, 4 or 8 pipettes and the includes a system to transfer samples from a microliter plate. The passive block contains 97 (385) pipettes (including the dummy tip) with the same measurements as the Spectrochip™ microchip. Each piezo electric pipette of the active/passive block is addressable with its own piezo electric dispensing parameters. With the passive block, system liquid is added simultaneously onto each of the 97 (385) positions of the chip.

## EXAMPLE 2

### Unsealed Nanoliter PCT Prior to MALDI-MS Analysis in a Single Tube Procedure

This example demonstrates that PCR products produced in an unsealed environment can be detected using matrix assisted laser desorption ionization (MALDI) mass spectrometry.

PCR is performed using pACT template, which is a pUC derivative harboring a 389 bp insert of human  $\beta$ -actin cDNA, biotinylated primer BAct2 d(bio-GAC TGA CTA CCT CAT GAA GAT CC) (SEQ ID No. 4) and non-biotinylated primer Aci4 d(GAA GCT GTA GCC GCG CTC GG) (SEQ ID No. 5). The reaction mix contains 5  $\mu$ l 10x PCR buffer (200 mM Tris-HCl (pH 8.75), 100 mM KCl, 100 mM  $(NH_4)_2SO_4$ , 20 mM  $MgSO_4$ , 1% Triton X-100, 1 mg BSA), a final concentration of 200  $\mu$ M of each dNTP, 10 pmol biotinylated primer, 100 pmol non-biotinylated primer, 2.5 Units Pfu DNA polymerase (Stratagene; La Jolla Calif.), 0.25 ng of template, and water to a final volume of 50  $\mu$ l. Amplification cycles were as follows: 30 cycles of 94° C. for 30 seconds, 65° C. for 30 seconds, and 72° C. for 30 seconds.

PCR was performed using a 1 channel NANO-PLOTTER pipetting device type NP1C (GeSim, Dresden Germany) modified with a heating element as described herein. This



pipetting device has an xyz table to move and dispense liquids from a piezo electric pipette. The pipette is connected to a pump system (diluter) to fill the pipette with liquid in the nanoliter scale from a microliter plate deposited on the z-table of the pipetting device. The other end of the diluter is connected to a reservoir that contains system liquid, for example, 50 ml ultrapure water. Valve settings in the diluter allow the bypass of system liquid to the piezo electric pipette. It is modified for use herein such that the target site (site to which liquid is dispensed) of the pipetting device has a 22W peltier heating/cooling element that is controlled by a programmable thermoelectric temperature controller LFI-3526 (Wavelength Electronic, Inc., Bozeman Mont.). Temperature is measured using a PT 500 type F3132 (Newport Electronics, Inc., Deckenpfromm Germany).

System liquid is heated to target temperature direct in the pipette to keep the temperature gradient between reaction liquid on the target and subsequently added system liquid zero. The reaction mix is deposited on two different positions on a Spectrochip™ microchip, which contains two hydrophilic modified positions. These target sites are surrounded by a hollow of black silicon containing a dense "forest" of 10 μm high needles. Due to the sharp edge between the target site and the hollow, the reaction volume grows only in the z-direction to a defined volume. Under the position where PCR occurs, a small magnet is mounted to capture paramagnetic beads, for example, streptavidin coated paramagnetic beads.

The second position (dummy position) is used to monitor the liquid lost due to evaporation. An inter digital array is set very close to this position to monitor drop size based on capacity measurement. The reaction mix and the dummy drop are kept constant during each PCR cycle by adjusting the dispensing frequency of the system liquid (see Example 1).

Five nl (25 drops) reaction mix are transferred from a microtiter plate onto the cooled (5° C.) Spectrochip™ microchip. The cooled trap is used to determine the initial drop height without evaporation. Once the mixture is deposited, the initial height of the reaction liquid is measured and PCR program was started. Following PCR, the Spectrochip™ microchip is cooled to 5° C. to trap the reaction liquid. In a first step after PCR, the piezo electric pipette transfers 10 nl streptavidin dynabeads (M-280) from a microtiter plate into the reaction mixture to capture PCR product. In a second step, the piezo electric pipette is used to flood the target area with 1 μl of 0.07 M ammonium citrate solution to rinse the reaction mix into the hollow. The washing step is repeated once. In a third step, the PCR product is denatured from the beads using ammonia at room temperature (RT). Thus, the target was adjusted to RT and the piezo electric pipette picks up 10% ammonia and transferred 50 nl onto the target site. In the fourth step, the target is cooled to 5° C. and the denatured PCR product is redissolved with 3 nl ultrapure water. In the last step, 6 nl matrix is added into the liquid PCR product, while again adjusting the target temperature to RT to obtain optimal crystallization. The SpectroChip™ support then is transferred into a mass spectrometer (Bruker/Sequenom, Germany), which allows automated measurement of the nanoliter reactions.

The reaction also can be monitored using a He—Ne laser, and can be performed using active and passive piezo electric pipettes as disclosed in Example 1. Using a 4 channel NANO-PLOTTER pipetting device, the last 4 steps as described in Example 1 can be reduced to 2 steps, including a first step, wherein pipette 1 contains the beads and pipette

2 contains the rinse liquid, and a second step, wherein pipette 3 contains the ammonia and pipette 4 contains the matrix. Utilizing two steps prevents the ammonia from evaporation and the matrix from crystallization.

### EXAMPLE 3

#### Unsealed Nanoliter Cycle Sequencing Prior to MALDI-MS Analysis in a One Well Procedure

This example demonstrates that cycle sequencing can be performed in a single well and the reaction product subsequently can be analyzed by MALDI-MS. Using the open method, no cover or sealing is used to prevent evaporation during the cycling program for the DNA sequencing reaction.

Reactions were performed essentially as described previously (see van den Boom et al., *Anal. Biochem.*; van den Boom et al., *J.B.B.M.* standard cycle sequencing paper; Koster et al., *Nature Biotech.*; Little et al., *Anal. Chem.* 69:4540-4546 (1997)). DNA sequencing of PCR products are amplified off-line on a microchip without sealing. Target DNA to be sequenced was amplified from genomic DNA or cDNA using a biotinylated primer. The corresponding PCR product was stored in a microtiter plate accessible for the piezoelectric pipette. PCR product can be placed on each position of a 96 Spectrochip™ support, thus allowing a series of cycle sequencing reactions to be performed according to a primer walking strategy, which yields the full sequence of the PCR product.

Sequence analysis is performed by a thermal cycled reaction using a short oligonucleotide primer complementary to the biotinylated PCR strand, a DNA polymerase and a sequencing nucleotide mix in a buffered reaction system. After completion of the reaction, the biotinylated PCR template strand is immobilized to streptavidin coated magnetic beads. The sequencing ladder that hybridizes to this strand is co-immobilized and can be separated from reaction components. After a washing step, the sequencing ladder is recovered from the streptavidin beads by denaturation. In a subsequent step, the sequence ladder can be mass analyzed directly from the SpectroChip™ support using a Bruker/Sequenom MALDI-TOF MS.

The following reagents were used: p53 specific PCR product: amplification primers d(CTGCTTGCCACAGGTCTC) (SEQ ID No. 6) and d(biotin-CACAGCAGGCCAGTGTGC) (SEQ ID No. 7) were targeted against exon 7 of the p53 gene. PCR was performed according to standard procedures using 10 pmol of forward primer and 6 pmol of biotinylated reverse primer. Sequencing primer specific for the exon 7 PCR product was d(gaggccatcctcacc) (SEQ ID No. 8). The cycle sequencing reaction mixture contained 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 1 mg/ml BSA, 3.5 mM MgCl<sub>2</sub>, 200 μM of each dNTP, 300 μM of sequencing primer, 1 Unit ThermoSequenase™ and 5 ng of p53 PCR product in a 10 μl total volume. M-280 streptavidin coated paramagnetic beads also were used. Cycle sequencing was performed using the following cycling conditions: 30 cycles of 94° C. for 10 seconds, 54° C. for 5 seconds, and 72° C. for 15 seconds.

Liquid handling was performed using a 2 channel NANO-PLOTTER pipetting device type NP1c (GeSim) modified with heating element. As noted above, this device contains an xyz table to move and dispense liquids from a piezoelectric pipette. The pipettes are connected to pump systems (diluters) to fill the pipettes with liquid in nanoliter volumes from a microliter plate deposited on the z-table of the

pipetting device. The other end of the diluters is connected to a reservoir containing the system liquid, 50 ml ultrapure water. Valve settings in the diluters allow to bypass system liquids to the piezo electric pipettes. The target site of the modified NANO-PLOTTER pipetting device has a 22W Peltier heating/cooling element controlled by a programmable thermoelectric temperature controller LFI-3526. Temperature is measured via PT 500 type F3132 (see Examples 1 and 2).

System liquid is heated to target temperature directly in the pipette to keep the temperature gradient between reaction liquid on the target and subsequently added system liquid zero. The reaction liquid is deposited on two different positions on a microchip support (e.g., a SpectroChip™ chip), which contains 2 hydrophilic modified positions (see Example 2). Under the position where the reaction takes place, a small magnet is mounted to capture paramagnetic beads. A second dummy position is included as described in Examples 1 and 2.

Five nl reaction mixtures are transferred from a microtiter plate onto a cooled (5° C.) Sequenom 97 position silicon SpectroChip™ microchip and the initial height of the reaction liquid is determined (see Examples 1 and 2). After the cycled sequencing reaction is completed, the SpectroChip™ microchip is cooled to 5° C. to trap the reaction liquid.

In a first step the piezo electric pipette transfers 10 nl streptavidin coated paramagnetic beads from the microtiter plate into the reaction mix to separate the sequencing ladder from reaction components. The second step is to flood the working area via the piezo electric pipette with 1 µl of 0.07 M ammonium citrate to rinse the reaction mix into the hollow; this step is repeated once. The third step is to denature the sequencing ladder from the beads with 10% ammonium hydroxide solution at room temperature. The target temperature is adjusted to RT and the piezo electric transfers 10 nl ammonia onto the working area. The fourth step is to cool the target again to 5° C. and redissolve the denatured sequencing products with nl ultrapure water. In the last step, 6 nl matrix is added into the liquid sequencing products while driving the target temperature again to RT to obtain optimal crystallization. The entire SpectroChip™ microchip is transferred into a Bruker/Sequenom mass spectrometer, which allows automated measurement from the nanoliter spots.

#### EXAMPLE 4

##### RNAse Digest of Ribo-Modified Oligonucleotides in a Single Tube Reaction

Liquid handling is performed using a modified 2 channel pipetting device type NP1c described in Example 1. The target site of the modified pipetting device has a 22W Peltier heating/cooling element controlled by a programmable thermoelectric temperature controller LFI-3526, and temperature is measured using PT 500 type F3132 (see Example 1).

System liquid is heated to target temperature directly in the pipette. The reaction liquid is deposited on two different positions on the SpectroChip™ microchip, which contains two modified hydrophobic positions to allow the reaction liquid to grow only in z-direction. One position was used as the reaction position and the second position is a dummy position to monitor the liquid loss due to evaporation (see Example 1).

Twenty nl (50 pmol) of a ribo-modified oligonucleotide is placed on the reaction position of the 96 position SpectroChip™ microchip, while the chip temperature is maintained

at 5° C. The cooled trap for the reaction liquid is used to determine the initial drop height without evaporation. From a microtiter plate, 20 nl of bovine pancreas RNase (Boehringer Mannheim) is added to the reaction position. The chip then is heated to a constant temperature of 37° C. for 15 min. Following completion of the reaction, the system is cooled to 4° C. and 25 nl of matrix is added, while driving the target temperature to RT to obtain optimal crystallization. The SpectroChip™ microchip is transferred into a Bruker/Sequenom mass spectrometer for automated analysis.

The detection of degraded fragments with a mass of 4524 Daltons (Da) indicates cleavage of the oligonucleotide at the modified site.

Sequence: CGAAXTCGAGCTCGGTACCC

Ribo-modification: X is rU.

#### EXAMPLE 5

##### Exonucleolytic Degradation of Oligonucleotides in a 12 Well Procedure

Liquid handling is performed on a modified 1 channel pipetting device type NP1c (GeSim, Dresden Germany). As noted, this pipetting device has a xyz table to move and dispense liquids from a piezoelectric pipette. The active piezoelectric pipette is mounted on the active block, i.e. the pipette is able to pick up samples from a microtiter plate prior to dispensing reagent onto a silicon chip support (a SpectroChip™ support). 97 pipettes with the same measurements as the SpectroChip™ support are connected to the passive block. With the passive block, system liquid can be added simultaneously to each of the 97 positions of the chip. The active and the passive pipettes are connected to their own pump system (diluter). Each piezo electric pipette of the active/passive block is addressable with its own piezo electric dispensing parameters in order to do pipette selection. The other ends of the diluters are connected to a reservoir which contains system liquid (50 mL ultrapure water). Valve settings in the diluters allow to bypass system liquid to the piezo electric pipette(s). The target site of the modified pipetting device has a 22W Peltier heating/cooling element which is controlled by a programmable thermoelectric temperature controller LFI-3526, and temperature is measured via PT 500 type F3132.

System liquid is heated to target temperature directly in the pipettes of the passive block to keep the temperature gradient between reaction liquid on the target and subsequently added system liquid zero. The SpectroChip™ microchip includes 97 modified hydrophobic positions, which allow the reaction liquid to grow only in z-direction; 96 positions can be used as reaction positions and the 97th position is a dummy position to monitor the liquid loss due to evaporation (see Example 1).

Using the active piezo electric pipette, 20 nl (50 pmol) of an oligonucleotide is placed on 12 positions of the 97 SpectroChip™ microchip, while the chip temperature is kept at 5° C., then 20 nl of snake venom phosphodiesterase (Boehringer Mannheim) is added to the 12 positions. The cooled trap for the reaction liquid is used to determine the initial drop high without evaporation. The SpectroChip™ microchip then is heated to a constant temperature of 37° C. In order to obtain a kinetic picture of the degradation reaction, the patches are allowed to dry sequentially two minutes after another. The drying process is monitored and controlled using a capacitor measurement system; system liquid replacement utilizes the passive piezo electric pipettes.

After all reactions are completed, the chip is cooled to 5° C. and the analyte is redissolved using the passive piezo electric pipette block. Matrix is added to the 12 positions, while driving the target temperature to RT to obtain the best crystallization results. The SpectroChip™ microchip then is transferred into a Bruker/Sequenom mass spectrometer which allows automated measurement from nanoliter spots, resulting in twelve spectra representing the whole oligonucleotide sequence.

#### EXAMPLE 6

##### Restriction Digest of PCT Products

Liquid handling is performed on the modified 1 channel NP1c dispensing device described in Example 5. The target site of the modified NANO-PLOTTER pipetting device has a 22W peltier heating/cooling element controlled by a programmable thermoelectric temperature controller LFI3526. Temperature is measured via PT 500 type F3132 (see Example 5).

System liquid was also heated to target temperature directly in the pipettes of the passive block to keep the temperature gradient between reaction liquid on the target and subsequently added system liquid zero. The SpectroChip™ microchip is as described in Example 5.

A portion of exon 4 of the human apolipoprotein-E gene is amplified by PCR in a conventional 96 well microtiter plate (see Little et al., *Int. J. Mass Spectrom. Ion Processes* 169/170:323-330 (1997)). Aliquots of each well (30 nl) are transferred to a 97 SpectroChip™ microchip with the active piezo electric pipette block while the chip temperature is maintained at 5° C., then 20 nl of CfoI and RsaI (Boehringer Mannheim) are added to each position. The cooled trap for the reaction liquid is used to determine the initial drop high without evaporation. The chip is heated to a constant temperature of 37° C. for 15 min, during which time the reaction volume is kept constant. After the reaction, the system is cooled to 4° C. and 25 nl of matrix is added to each position, while driving the target temperature to RT for optimal crystallization. The entire SpectroChip™ microchip is transferred into a Bruker/Sequenom mass spectrometer for automated analysis of the nanoliter spots.

With respect to the genotype of the genomic DNA used as template in the PCR reactions, different fragment pattern are observed. The genotype epsilon 3 results, for example, in fragments having molecular masses of 6749 Da, 7521 Da, 14858 Da, 18839 Da, 29708 Da and 33331 Da.

#### EXAMPLE 7

##### Nanoliter Liquid Handling System For Real Time DNA Sequencing By Detection of Pyrophosphate Release

Sequencing by synthesis (pyrophosphate sequencing) is performed by detecting DNA polymerase activity by an enzymatic luminometric assay, which determines the amount of inorganic pyrophosphate ("ELIDA assay"; see Ronaghi et al., *Anal. Biochem.* 267:65-71 (1999); Ronaghi et al., *Biotechniques* 25:876-878, 880-882, and 884 (1998); Ronaghi et al., *Science* 281:363-365 (1998); Ronaghi et al., *Anal. Biochem.* 242:84-89 (1996); Nyren, *Anal. Biochem.* 167:235-238 (1987)).

Pyrophosphate sequencing is performed as follows. Either a synthetic template or a PCR product is immobilized onto a solid support. Immobilization can be performed employing standard procedures such as the streptavidin biotin system or

SIAB chemistry, as disclosed herein. In case of PCR product sequencing, the double stranded PCR product is denatured, for example, by alkaline treatment, prior to the sequencing reaction.

An oligonucleotide primer is annealed to the immobilized template strand. A DNA polymerase and one deoxynucleotide triphosphate in a buffered system are added and incorporation of the nucleotide is monitored by the release of inorganic pyrophosphate. The added nucleotide is incorporated by the polymerase only if it is complementary to the corresponding position in the template sequence. The release of pyrophosphate, a result of the incorporation of the nucleotide triphosphate, is monitored as follows: inorganic pyrophosphate is converted to ATP via the ATP sulfurylase, and the level of present ATP is monitored by the firefly luciferase system.

In the sequencing process, the addition of nucleotide triphosphates is performed in a stepwise manner. The reaction is allowed to proceed for a certain time, then the reaction mix is separated from the solid support and, therefore, from the immobilized template primer system, and further washing is performed. Following washing, the next nucleotide triphosphate is added as part of a reaction mix, containing all necessary enzymes and buffers and monitoring is performed as above. Repeating the additions with all four possible nucleotide triphosphates in a cyclic manner allows stepwise (base for base) sequencing of the template strand.

Liquid handling is performed on a modified 8 channel pipetting device type NP1c from (GeSim, Dresden Germany). As noted above, this pipetting device includes an xyz table to move and dispense liquids from a piezo electric pipette. The active piezo electric pipettes are mounted on the active block, i.e. the pipettes are able to pick up samples from a microtiter plate prior to dispensing reagent onto a support, such as a microchip type support SpectroChip™ microchip. 97 pipettes with the same measurements as the SpectroChip™ microchip are connected to the massive block. With the passive block system liquid can be added simultaneously onto each of the 97 positions of the chip.

The active and the passive pipettes are connected to their own pump system (diluter). Each piezo electric pipette of the active/passive block is addressable with its own piezo electric dispensing parameters in order to do pipette selection. The other ends of the diluters are connected to a reservoir which contains system liquid (50 ml ultrapure water). Valve settings in the diluters allow to bypass system liquid to the piezo electric pipette(s).

The target site of the modified pipetting device has a 22W Peltier heating/cooling element which is controlled by a programmable thermoelectric temperature controller LFI-3526. Temperature is measured via PT 500 type F3132. System liquid is heated to target temperature directly in the pipettes of the passive block to keep the temperature gradient between reaction liquid on the target and subsequently added system liquid zero. The SpectroChip™ microchip has 97 modified hydrophobic positions, which allow the reaction liquid to grow only in the z-direction; 96 positions can be used as reaction positions, one of which is used to monitor the reaction using, for example, a fiber optic set very close to the reaction position. The fiber optic is connected to a

photomultiplier to convert the luminescence signal to an electric signal. A bandpass filter is placed between fiber optic and photomultiplier. The 97th position (dummy position) is used to monitor the liquid loss due to evaporation as described in Example 1. The whole system is covered and darkened to reduce background effect while obtaining the fluorescence signal.

The universal reaction mix contains 0.1 M Tris-acetate (pH 7.75), 2 mM EDTA, 10 mM magnesium acetate, 0.1% BSA, 1 mM dithiothreitol, 5  $\mu$ M adenosine 5'-phosphosulfate (APS), 0.4 mg/ml polyvinylpyrrolidone, 100  $\mu$ g/ml D-luciferin (bioOrbit, Finland), 4  $\mu$ g/ml L-Luciferin (BioOrbit, Finland), 0.3 U/ml ATP sulfurylase (ATP:sulfate adenylyltransferase; EC 2.7.7.4; Sigma Chemical Co., St. Louis, Mo.), purified luciferase (Sigma) to yield a useable luminometric response, and 2.5 U DNA polymerase (Sequenase 2.0, Amersham). Nucleotide triphosphate mixes include 4 separate mixes containing an aqueous solution of either 50  $\mu$ M S-dATP, dCTP, dGTP and dTTP. Washing solution I is 10 mM Tris-HCl (pH 7.5), 0.25 M NaCl, 0.1% Tween 20. Washing solution II is 10 mM Tris-acetate (pH 7.5). Primer solution is an aqueous solution of 200  $\mu$ M of sequencing primer

PCR product is amplified from target DNA using a 5'-thiolated primer, amplification products are stored in a MTP and supplied to the nanoliquid handling device. PCR product is pipetted from the MTP on the chip using the piezoelectric pipette of the nanoliquid handling system. PCR product is immobilized on the chip surface via SIAB chemistry, and is denatured upon addition of 5 nl of 100 mM NaOH using the piezoelectric pipette of the nanoliquid handling system. Remaining immobilized single stranded PCR product is washed with 10 mM Tris-HCl (pH 8.0) twice.

Sequencing reactions are performed using an 8-channel piezoelectric pipette for automated performance of the cycled stepwise nucleotide addition. One pipette is filled with water, one with the reaction mix, four pipettes are necessary to contain the separate nucleotides and two pipettes contain the washing solutions. Sequencing is performed as follows:

1. The piezoelectric pipette of the nanoliquid handling system fills with the sequencing primer solution of the MTP and adds it to the immobilized template strand on the chip.
2. All 8 pipettes of the head then are filled with the respective solutions from the supply MTP.
3. The chip well is heated to 95° C. to denature secondary structure of the PCR product and is slowly cooled to RT to allow annealing of the sequencing primer. During this thermal step, evaporation is prevented by sequential addition of water droplets from a piezoelectric pipette Nr. 1.
4. The primer template hybrid is washed by addition of the washing solution I from a piezoelectric pipette Nr.2.
5. The reaction mix is added to the primer template system using the corresponding piezoelectric pipette Nr.3.
6. Upon addition of the first nucleotide (SdATP) using piezoelectric pipette Nr. 4, the incorporation reaction is initiated and luminescence is monitored; the reaction is allowed to proceed for 10 seconds, during which time the reaction volume is maintained by sequential addition of water through pipette 1. Incorporation is identified by increased luminescence. Intensities are analyzed to determine the number of sequential incorporation of the respective nucleotide.

7. The reaction is stopped by rinsing the immobilized DNA with washing solution I from pipette 2.
  8. The immobilized DNA is washed by addition of washing solution II from pipette 8.
  9. Reaction mix is again deposited onto the chip by pipette 3.
  10. Upon addition of the second nucleotide (dCTP) using piezoelectric pipette Nr. 5, the incorporation reaction is started and the luminescence is monitored; the reaction is allowed to proceed for 10 seconds, during which time the reaction volume is kept constant by sequential addition of water through pipette 1. The incorporation is identified by increased luminescence. intensities are analyzed to determine the number of sequential incorporation of the respective nucleotide.
  11. The reaction is stopped by rinsing the immobilized DNA with washing solution I from pipette 2.
  12. The immobilized DNA is washed by addition of washing solution II from pipette 8.
  13. Reaction mix is again deposited onto the chip by pipette 3.
  14. Upon addition of the third nucleotide (dGTP) using piezoelectric pipette Nr. 6, the incorporation reaction is started and the luminescence is monitored; the reaction is allowed to proceed for 10 seconds, during which time the reaction volume is kept constant by sequential addition of water through pipette 1. The incorporation is identified by increased luminescence. Intensities are analyzed to determine the number of sequential incorporation of the respective nucleotide.
  15. The reaction is stopped by rinsing the immobilized DNA with washing solution I from pipette 2.
  16. The immobilized DNA is washed by addition of washing solution II from pipette 8.
  17. Reaction mix is again deposited onto the chip by pipette 3.
  18. Upon addition of the fourth nucleotide (dTTP) using piezoelectric pipette Nr. 7, the incorporation reaction is started and the luminescence is monitored; the reaction is allowed to proceed for 10 seconds, during which time reaction volume is kept constant by sequential addition of water through pipette 1. The incorporation is identified by increased luminescence. Intensities are analyzed to determine the number of sequential incorporation of the respective nucleotide.
  19. The reaction is stopped by rinsing the immobilized DNA with washing solution I from pipette 2.
  20. The immobilized DNA is washed by addition of washing solution II from pipette 8.
  21. Reaction mix is again deposited onto the chip by pipette 3.
  22. The reaction scheme proceeds in a cycled manner by looping back to step 6.
- While the invention has been described with some specificity, modifications apparent to those with ordinary skill in the art may be made without departing from the scope of the invention. Since such modifications will be apparent to those of skill in the art, it is intended that this invention be limited only by the scope of the appended claims.

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<223> OTHER INFORMATION: Forward PCR primer for human B-actin gene

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25

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25

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25

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<223> OTHER INFORMATION: Forward PCR primer for 389 bp human B-actin  
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&lt;223&gt; OTHER INFORMATION: Biotinylation at the 5'-end

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23

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&lt;220&gt; FEATURE:

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&lt;220&gt; FEATURE:

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&lt;222&gt; LOCATION: (1)..(20)

&lt;223&gt; OTHER INFORMATION: Reverse PCR primer for 389 bp human B-actin cDNA insert

&lt;400&gt; SEQUENCE: 5

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20

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&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: primer\_bind

&lt;222&gt; LOCATION: (1)..(18)

&lt;223&gt; OTHER INFORMATION: Forward amplification primer directed against exon 7 of human p53 gene

&lt;400&gt; SEQUENCE: 6

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18

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&lt;223&gt; OTHER INFORMATION: Reverse amplification primer directed against exon 7 of the human p53 gene

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&lt;222&gt; LOCATION: (1)

&lt;223&gt; OTHER INFORMATION: 5'-biotinylated primer

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19

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&lt;221&gt; NAME/KEY: primer\_bind

&lt;222&gt; LOCATION: (1)..(16)

&lt;223&gt; OTHER INFORMATION: Sequencing primer for exon 7 of human p53 gene

-continued

&lt;400&gt; SEQUENCE: 8

gaggcccatc ctcacc

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What is claimed is:

1. An open system for performing a reaction while maintaining constant concentrations of reactants, comprising:
  - a support for performing the reaction in an open environment, wherein:
    - the support is for containing or retaining biopolymers or biological particles; and
    - the reaction is a submicroliter volume reaction;
    - a nanoliter dispensing pipette for dispensing an amount of a liquid onto the surface of the support;
    - a temperature controlling device for regulating the temperature of the support; and
  - means for controlling the amount of liquid dispensed, wherein:
    - the amount of liquid dispensed corresponds to the amount of liquid that evaporates from the support to thereby maintain the concentration of reactants in the reaction; and
    - the system is not sealed.
2. The open system of claim 1, wherein the controlling means comprises software that calculates the amount of liquid that evaporates and signals the dispensing pipette to deliver an amount of liquid that corresponds to the amount that evaporates.
3. The open system of claim 1, wherein the controlling means comprises manual input.
4. The open system of claim 1, further comprising a means for determining the temperature of a liquid on the support.
5. The open system of claim 1, wherein the support comprises a bead, pin, comb, wafer, well or microchip.
6. An open system for performing a reaction, comprising:
  - a support functionalized for linking a biopolymer or biological particle and for performing the reaction, wherein the support comprises a bead, pin, comb, wafer, well or microchip;
  - a nanoliter dispensing pipette for dispensing an amount of a liquid onto the surface of the support;
  - a temperature controlling device for regulating the temperature of the support; and
  - means for controlling the amount of liquid dispensed, wherein the amount of liquid dispensed corresponds to the amount of liquid that evaporates from the support, wherein the system is not sealed.
7. The open system of claim 1, wherein the reaction is a submicroliter reaction.
8. A system, comprising:
  - a solid support having a target site for retaining or containing a liquid;
  - a liquid dispensing system for dispensing a liquid to the target site;
  - a temperature controlling system, which regulates the temperature of the solid support; and
  - an interface for indicating an amount of liquid to be dispensed to the target site, wherein the amount of liquid to be dispensed corresponds to an amount of liquid that evaporates from the target site, wherein:
    - the interface monitors the level of a liquid on the target site; and
    - the interface comprises a light source and a photometer.
9. A system, comprising:
  - a solid support having a target site for retaining or containing a liquid;
  - a liquid dispensing system for dispensing a liquid to the target site;
  - a temperature controlling system, which regulates the temperature of the solid support; and
  - an interface for indicating an amount of liquid to be dispensed to the target site, wherein the amount of liquid to be dispensed corresponds to an amount of liquid that evaporates from the target site, wherein the interface monitors the concentration of a component in the liquid.
10. The system of claim 8, wherein the temperature controlling system comprises a Peltier element.
11. The system of claim 8, further comprising a detection system.
12. A system comprising:
  - means for dispensing a liquid;
  - means for containing a reaction volume comprising a microchip or glass slide, wherein the microchip or glass slide comprises on a surface, an array of hydrophilic regions and adjacent hydrophobic regions;
  - means for controlling the temperature of the reaction volume containing means; and
  - means for regulating an amount of liquid dispensed from the liquid dispensing means based on the temperature of the reaction volume containing means.
13. A method for performing a reaction in an unsealed environment, comprising the steps of:
  - a) dispensing a predetermined submicroliter volume of liquid onto a target site on a surface of a support;
  - b) determining an amount or rate of evaporation of the liquid from the target site; and
  - c) dispensing a further amount of liquid to the target site, wherein the further amount dispensed corresponds to the amount of liquid that evaporates from the target site, thereby maintaining the reaction volumes and concentration of reactant at a predetermined volume throughout the course of the reaction.
14. The method of claim 13, wherein the reaction is performed in a submicroliter volume.
15. The method of claim 13, wherein the amount or rate of evaporation is determined by monitoring the temperature of the target site and calculating the amount or rate of evaporation.
16. A method for performing a reaction in an unsealed environment, comprising the steps of:
  - a) dispensing a predetermined submicroliter volume of liquid onto a target site on a surface of a support;
  - b) determining an amount or rate of evaporation of the liquid from the target site, wherein the amount or rate of evaporation is determined by monitoring the conductance of the liquid at the target site; and

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- c) dispensing a further amount of liquid to the target site, wherein the further amount dispensed corresponds to the amount of liquid that evaporates from the target site, thereby maintaining the reaction volume at a predetermined volume throughout the course of the reaction.
17. A method for performing a reaction in an unsealed environment, comprising the steps of:
- a) dispensing a predetermined submicroliter volume of liquid onto a target site on a surface of a support;
  - b) determining an amount or rate of evaporation of the liquid from the target site; and
  - c) dispensing a further amount of liquid to the target site, wherein the further amount dispensed corresponds to the amount of liquid that evaporates from the target site, thereby maintaining the reaction volume at a predetermined volume throughout the course of the reaction, wherein the reaction is a nucleic acid amplification reaction.
18. The method of claim 13, wherein the reaction is one of a plurality of reactions.
19. A method for performing a reaction in an unsealed environment, comprising the steps of:
- a) dispensing a predetermined submicroliter volume of liquid onto a target site on a surface of a support;
  - b) determining an amount or rate of evaporation of the liquid from the target site; and
  - c) dispensing a further amount of liquid to the target site, wherein the further amount dispensed corresponds to the amount of liquid that evaporates from the target site, thereby maintaining the reaction volume at a predetermined volume throughout the course of the reaction, wherein:
- the reaction is one of a plurality of reactions, and the plurality of reactions are performed at a single target site.
20. The method of claim 19, wherein the reactions are performed simultaneously.
21. The method of claim 20, which is a multiplex reaction.
22. The method of claim 18, wherein each reaction in the plurality is performed at a single target site.
23. A method for performing a reaction in an unsealed environment, comprising the steps of:
- a) dispensing a predetermined submicroliter volume of liquid onto a target site on a surface of a support;
  - b) determining an amount or rate of evaporation of the liquid from the target site; and
  - c) dispensing a further amount of liquid to the target site, wherein the further amount dispensed corresponds to the amount of liquid that evaporates from the target site, thereby maintaining the reaction volume at a predetermined volume throughout the course of the reaction, wherein:
- the reaction is one of a plurality of reactions; each reaction in the plurality is performed at a single target site; and the method comprises synthesis of a combinatorial library.
24. A method for performing a reaction in an unsealed environment, comprising the steps of:
- a) dispensing a predetermined submicroliter volume of liquid onto a target site on a surface of a support;
  - b) determining an amount or rate of evaporation of the liquid from the target site; and
  - c) dispensing a further amount of liquid to the target site, wherein the further amount dispensed corresponds to

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- the amount of liquid that evaporates from the target site, thereby maintaining the reaction volume at a predetermined volume throughout the course of the reaction; and
- d) detecting a component of the reaction, detecting a component of the reaction.
25. A method for maintaining a volume of a reaction mixture on a solid support in an unsealed environment, comprising the steps of:
- a) determining the amount of liquid that evaporates from the reaction mixture; and
  - b) dispensing into the reaction mixture an amount of liquid that corresponds to the amount of liquid that evaporates, thereby maintaining the volume of the reaction mixture, wherein the reaction mixture comprises a biopolymer.
26. The method of claim 25, wherein the reaction mixture comprises a biopolymer sequencing reaction.
27. The method of claim 25, wherein the biopolymer is a polynucleotide.
28. The method of claim 26, wherein the sequencing reaction comprises a chemical cleavage reaction.
29. The method of claim 26, wherein the sequencing reaction comprises an enzymatic reaction.
30. The method of claim 29, wherein the enzymatic reaction is a chain termination reaction comprising a polymerase and a chain terminating nucleoside triphosphate.
31. The method of claim 29, wherein the enzymatic reaction is an exonuclease reaction.
32. The method of claim 26, wherein the biopolymer is a polypeptide.
33. The method of claim 32, wherein the sequencing reaction is selected from the group consisting of a chemical sequencing reaction and an enzymatic sequencing reaction.
34. The method of claim 26, wherein the biopolymer is selected from the group consisting of a carbohydrate, a glycoprotein, a proteoglycan and a lipid.
35. The method of claim 25, wherein the reaction mixture comprises a biopolymer synthesis reaction.
36. The method of claim 35, wherein the biopolymer is a polynucleotide.
37. The method of claim 36, wherein the synthesis reaction is a chemical synthesis reaction.
38. The method of claim 36, wherein the synthesis reaction is an enzymatic synthesis reaction, comprising a polymerase.
39. The method of claim 38, wherein the biopolymer and the polymerase are selected from the group consisting of:
- a) a ribonucleic acid and an RNA dependent RNA polymerase;
  - a) a ribonucleic acid and an RNA dependent DNA polymerase; and
  - a) a deoxyribonucleic acid and a DNA dependent DNA polymerase.
40. The method of claim 38, comprising a ribonucleic acid, an RNA dependent DNA polymerase, and a DNA dependent DNA polymerase.
41. The method of claim 38, wherein the enzymatic synthesis reaction is a polymerase chain reaction, further comprising a set of primers.
42. The method of claim 41, wherein a primer of the set of primers comprises a peptide nucleic acid sequence.
43. The method of claim 35, wherein the biopolymer is a polypeptide.
44. The method of claim 35, wherein the biopolymer is selected from the group consisting of a carbohydrate, a glycoprotein, a proteoglycan and a lipid.



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45. The method of claim 35, wherein the biopolymer is a polynucleotide and the reaction mixture comprises a primer extension reaction.

46. The method of claim 45, wherein the primer extension reaction is selected from the group consisting of:

- competitive oligonucleotide single base extension;
- primer oligo base extension (PROBE);
- loop-PROBE; and
- telomeric repeat amplification.

47. The method of claim 25, wherein the reaction mixture comprises an assay for detecting the presence of the biopolymer.

48. The method of claim 47, wherein the biopolymer is obtained from a biological sample.

49. The method of claim 48, wherein the method comprises a diagnostic method for detecting the presence of or predisposition to a genetic disease or condition.

50. The method of claim 48, wherein the method comprises determining a genotype.

51. The method of claim 25, wherein the biopolymer is immobilized to the solid support.

52. The method of claim 47, comprising detecting the biopolymer by identifying a detector oligonucleotide that has hybridized to the biopolymer.

53. The method of claim 52, wherein the detector oligonucleotide comprises a peptide nucleic acid sequence.

54. A method for performing a plurality of reactions in an unsealed environments, comprising the steps of:

- a) providing a plurality of reaction mixtures, wherein each reaction in the plurality is at a target site on a surface of a solid support;
- b) monitoring the amount of liquid that evaporates from reaction mixtures in the plurality; and
- c) dispensing into the reaction mixtures an amount of liquid that corresponds to the amount of liquid that evaporates from the reaction mixtures, thereby maintaining the volume of the reaction mixtures in the plurality at a predetermined volume, wherein one or more of the reaction mixtures in the plurality comprises one or more biopolymers.

55. The method of claim 54, wherein a biopolymer present in a reaction mixture is immobilized to the solid support.

56. The method of claim 54, wherein the solid support comprises a microchip.

57. The method of claim 56, wherein the plurality of reaction mixtures is present in an array on the microchip.

58. A method for performing a plurality of reactions in an unsealed environments, comprising the steps of:

- a) providing a plurality of reaction mixtures, wherein each reaction in the plurality is at a target site on a surface of a solid support;
- b) monitoring the amount of liquid that evaporates from reaction mixtures in the plurality;

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c) dispensing into the reaction mixtures an amount of liquid that corresponds to the amount of liquid that evaporates from the reaction mixtures, thereby maintaining the volume of the reaction mixtures in the plurality at a predetermined volume; and

d) detecting a component of a reaction mixture in the plurality.

59. The method of claim 58, wherein the component is a biopolymer.

60. The method of claim 59, wherein the biopolymer is detected directly.

61. The method of claim 59, wherein the biopolymer is detected indirectly.

62. The method of claim 61, wherein the biopolymer is a polynucleotide, which is detected by detecting an amplification product produced from the polynucleotide.

63. The method of claim 61, wherein the biopolymer is detected by identifying a reagent that binds specifically to the biopolymer.

64. The method of claim 63, wherein the biopolymer is a polynucleotide and the reagent is an oligonucleotide that hybridizes specifically to the polynucleotide.

65. The method of claim 64, wherein the oligonucleotide is a primer, which has been extended due to the activity of a polymerase.

66. The method of claim 63, wherein the biopolymer is a first polypeptide and the reagent is a second polypeptide that binds specifically to the first polypeptide.

67. The method of claim 63, wherein the second polypeptide is an antibody.

68. The method of claim 58, wherein the component is detected by a method selected from mass spectrometry, spectrophotometry, and capillary electrophoresis.

69. A method for performing a plurality of reactions in an unsealed environment, comprising:

- a) providing a plurality of reaction mixtures, wherein each reaction in the plurality is performed at a target site on a surface of a solid support that contains or retains the reaction mixtures;
- b) monitoring the amount of liquid that evaporates from reaction mixtures in the plurality; and
- c) dispensing into the reaction mixtures an amount of liquid that corresponds to the amount of liquid that evaporates from the reaction mixtures, thereby maintaining the volume of the reaction mixtures in the plurality at a predetermined volume, whereby the concentrations of reactants in the reaction mixtures remain constant.

70. The system of claim 14, wherein the temperature controlling system comprises a Peltier element.

71. The system of claim 14, further comprising a detection system.

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US99/15968 (22) International Filing Date: 14 July 1999 (14.07.99) (30) Priority Data: 09/115,455 14 July 1998 (14.07.98) US (71) Applicant: ZYOMYX, INC. [US/US]; 3912 Trust Way, Hayward, CA 94545 (US). (72) Inventors: WAGNER, Peter; 2211 Village Court #7, Belmont, CA 94002 (US). NOCK, Steffen; 3629 Glenwood Avenue, Redwood City, CA 94062 (US). AULT-RICHE, Dana; 972 Cajon Way, Palo Alto, CA 94303 (US). ITIN, Christian; 315 Waverley Street #3, Menlo Park, CA 94025 (US). (74) Agents: CHOW, Y., Ping et al.; Heller Ehrman White & McAuliffe, 525 University Avenue, Palo Alto, CA 94301-1900 (US).		(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>

(54) Title: ARRAYS OF PROTEIN-CAPTURE AGENTS AND METHODS OF USE THEREOF

## (57) Abstract

Arrays of protein-capture agents useful for the simultaneous detection of a plurality of proteins which are the expression products, or fragments thereof, of a cell or population of cells in an organism are provided. A variety of antibody arrays, in particular, are described. Methods of both making and using the arrays of protein-capture agents are also disclosed. The invention arrays are particularly useful for various proteomics applications including assessing patterns of protein expression and modification in cells.

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## ARRAYS OF PROTEIN-CAPTURE AGENTS AND METHODS OF USE THEREOF

### BACKGROUND OF THE INVENTION

#### a) Field of the Invention

The present invention relates generally to arrays of protein-capture agents and methods for the parallel detection and analysis of up to a large number of proteins in a sample. More specifically, the present invention relates to proteomics and the measurement of gene activity at the protein level in cells.

#### b) Description of Related Art

Although attempts to evaluate gene activity and to decipher biological processes including those of disease processes and drug effects have traditionally focused on genomics, proteomics offers a more direct and promising look at the biological functions of a cell. Proteomics involves the qualitative and quantitative measurement of gene activity by detecting and quantitating expression at the protein level, rather than at the messenger RNA level. Proteomics also involves the study of non-genome encoded events including the post-translational modification of proteins, interactions between proteins, and the location of proteins within the cell. The structure, function, or level of activity of the proteins expressed by a cell are also of interest. Essentially, proteomics involves the study of part or all of the status of the total protein contained within or secreted by a cell.

The study of gene expression at the protein level is important because many of the most important cellular processes are regulated by the protein status of the cell, not by the status of gene expression. Also, the protein content of a cell is highly relevant to drug discovery efforts since most drugs are designed to be active against protein targets.

Measuring the mRNA abundances of a cell provides only an indirect and incomplete assessment of the protein content of a cell. The level of active protein that is produced in a cell is often determined by factors other than the amount of

mRNA produced. For instance, both protein maturation and protein degradation are actively controlled in the cell and a protein's activity status can be regulated by post-translational modifications. Studies comparing mRNA transcript abundances to protein abundances have found only a limited correlation (coefficient of about 0.43-0.48) between the two (Anderson and Anderson, *Electrophoresis*, 19:1853-1861, 1998). Furthermore, the extreme lability of RNA in samples due to chemical and enzymatic degradation makes the evaluation of genetic expression at the protein level more practical than at the mRNA level. Current technologies for the analysis of proteomes are based on a variety of protein separation techniques followed by identification of the separated proteins. The most popular method is based on 2D-gel electrophoresis followed by "in-gel" proteolytic digestion and mass spectroscopy. Alternatively, Edman methods may be used for the sequencing. This 2D-gel technique requires large sample sizes, is time consuming, and is currently limited in its ability to reproducibly resolve a significant fraction of the proteins expressed by a human cell. Techniques involving some large-format 2D-gels can produce gels which separate a larger number of proteins than traditional 2D-gel techniques, but reproducibility is still poor and over 95% of the spots cannot be sequenced due to limitations with respect to sensitivity of the available sequencing techniques. The electrophoretic techniques are also plagued by a bias towards proteins of high abundance. Standard assays for the presence of an analyte in a solution, such as those commonly used for diagnostics, for instance, involve the use of an antibody which has been raised against the targeted antigen. Multianalyte assays known in the art involve the use of multiple antibodies and are directed towards assaying for multiple analytes. However, these multianalyte assays have not been directed towards assaying the total or partial protein content of a cell or cell population. Furthermore, sample sizes required to adapt such standard antibody assay approaches to the analysis of even a fraction of the estimated 100,000 or more different proteins of a human cell and their various modified states are prohibitively large. Automation and/or miniaturization of antibody assays are



required if large numbers of proteins are to be assayed simultaneously. Materials, surface coatings, and detection methods used for macroscopic immunoassays and affinity purification are not readily transferable to the formation or fabrication of miniaturized protein arrays.

Miniaturized DNA chip technologies have been developed (for example, see U.S. Patent Nos. 5,412,087, 5,445,934, and 5,744,305) and are currently being exploited for the screening of gene expression at the mRNA level. These chips can be used to determine which genes are expressed by different types of cells and in response to different conditions. However, DNA biochip technology is not transferable to protein-binding assays such as antibody assays because the chemistries and materials used for DNA biochips are not readily transferable to use with proteins. Nucleic acids such as DNA withstand temperatures up to 100°C, can be dried and re-hydrated without loss of activity, and can be bound physically or chemically directly to organic adhesion layers supported by materials such as glass while maintaining their activity. In contrast, proteins such as antibodies are preferably kept hydrated and at ambient temperatures are sensitive to the physical and chemical properties of the support materials. Therefore, maintaining protein activity at the liquid-solid interface requires entirely different immobilization strategies than those used for nucleic acids. The proper orientation of the antibody or other protein at the interface is desirable to ensure accessibility of their active sites with interacting molecules. With miniaturization of the chip and decreased feature sizes, the ratio of accessible to non-accessible and the ratio of active to inactive antibodies or proteins become increasingly relevant and important.

Thus, there is a need for the ability to assay in parallel a multitude of proteins expressed by a cell or a population of cells in an organism, including up to the total set of proteins expressed by the cell or cells.

#### SUMMARY OF THE INVENTION

The present invention is directed to arrays of protein-capture agents and methods of use thereof that satisfy the need to assay in parallel a multitude of proteins

expressed by a cell or population of cells in an organism, including up to the total protein content of a cell.

In one embodiment, the present invention provides an array of protein-capture agents comprising: a substrate; at least one organic thinfilm covering some or all of the surface of the substrate; and a plurality of patches arranged in discrete, known regions on the portions of the substrate surface covered by organic thinfilm, wherein (i) each patch comprises protein-capture agents immobilized on the organic thinfilm, where the protein-capture agents of a given patch are capable of binding a particular expression product, or a fragment thereof, of a cell or population of cells in an organism; and (ii) the array comprises a plurality of different protein-capture agents, each of which is capable of binding a different expression product, or fragment thereof, of the cell or population of cells in the organism.

In a second embodiment, the invention provides an array of bound proteins which comprises both the array of protein-capture agents of the invention and a plurality of different proteins which are expression products, or fragments thereof, of a cell or population of cells in an organism, where each of the different proteins is bound to a protein-capture agent on a separate patch of the array. Methods of using the arrays of protein-capture agents of the invention are also provided. In one embodiment of the invention, a method of assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism, is provided which comprises first delivering the sample to the array of protein-capture agents of the invention under conditions suitable for protein binding, wherein each of the proteins being assayed is a binding partner of the protein-capture agent of at least one patch on the array. The final step comprises detecting, either directly or indirectly, for the presence or amount of protein bound to each patch of the array. This method optionally further comprises the step of further characterizing the proteins bound to at least one patch of the array.

In another embodiment of the invention, a method for determining the protein expression pattern of a cell or a population of cells in an organism is provided which comprises first delivering a sample containing the expression products, or fragments thereof, of the cell or population of cells to the array of protein-capture agents of the invention under conditions suitable for protein binding. The final step comprises detecting, either directly or indirectly, for the presence or amount of protein bound to each patch of the array. In an alternative embodiment, a similar method for comparing the protein expression patterns of two cells or populations of cells is also provided.

In still another embodiment of the invention, an alternative method of assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism is provided. The method of this embodiment comprises first contacting the sample with an array of spatially distinct patches of different protein-capture agents under conditions suitable for protein binding, wherein each of the proteins being assayed is a binding partner of the protein-capture agent of at least one patch on the array. The last step of the method involves detecting, either directly or indirectly, for the presence or amount of protein bound to each patch of the array.

In a still further embodiment, a method of producing an array of protein-capture agents is provided which comprises the following steps: selecting protein-capture agents from a library of protein-capture agents, wherein the protein-capture agents are selected by their binding affinity to the proteins from a cellular extract or body fluid; producing a plurality of purified samples of the selected protein-capture agents; and immobilizing the protein-capture agent of each different purified sample onto an organic thinfilm on a separate patch on the substrate surface to form a plurality of patches of protein-capture agents on discrete, known regions of the surface of a substrate.

In an alternative embodiment, the invention provides a method for producing an array of protein-capture agents which comprises a first step of selecting protein-capture agents from a library of protein-capture agents, wherein the protein-

capture agents are selected by their binding affinity to proteins which are the expression products, or fragments thereof, of a cDNA expression library. The second step of the method comprises producing a plurality of purified samples of the protein-capture agents selected in the first step. The third step comprises immobilizing the protein-capture agent of each different purified sample onto an organic thinfilm on a separate patch on the substrate surface to form a plurality of patches of protein-capture agents on discrete, known regions of the surface of a substrate.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the top view of an array of patches reactive towards protein-capture agents.

Figure 2 shows the cross section of an individual patch of the array of Figure 1.

Figure 3 shows the cross section of a row of monolayer-covered patches of the array of Figure 1.

Figure 4 shows a thiolreactive monolayer on a substrate.

Figure 5 shows an aminoreactive monolayer on a coated substrate.

Figure 6 shows the immobilization of a protein-capture agent on a monolayer-coated substrate via an affinity tag.

Figure 7 shows the immobilization of a protein-capture agent on a monolayer-coated substrate via an affinity tag and an adaptor.

Figure 8 shows a schematic of a fluorescence detection unit which may be used to monitor binding of proteins by the protein-capture agents of the array.

Figure 9 shows a schematic of an ellipsometric detection unit which may be used to monitor binding of proteins by the protein-capture agents of the array.

#### DETAILED DESCRIPTION OF THE INVENTION

A variety of arrays of protein-capture agents and methods useful for multianalyte analyses and analyses of protein expression and modification in cells are provided by the present invention.

(a) Definitions.

The term "protein-capture agent" means a molecule or a multi-molecular complex which can bind a protein to itself. Protein-capture agents preferably bind their binding partners in a substantially specific manner. Protein-capture agents with a dissociation constant ( $K_D$ ) of less than about  $10^{-6}$  are preferred. The protein-capture agent will most typically be a biomolecule such as a protein or a polynucleotide. The biomolecule may optionally be a naturally occurring, recombinant, or synthetic biomolecule. Antibodies or antibody fragments are highly suitable as protein-capture agents. Antigens may also serve as protein-capture agents, since they are capable of binding antibodies. A receptor which binds a protein ligand is another example of a possible protein-capture agent. For instance, protein-capture agents are understood not to be limited to agents which only interact with their binding partners through noncovalent interactions.

Protein-capture agents may also optionally become covalently attached to proteins which they bind. For instance, the protein-capture agent may be photocrosslinked to its binding partner following binding.

The term "binding partner" means a protein which is bound by a particular protein-capture agent, preferably in a substantially specific manner. In some cases, the protein-capture agent may be a cellular or extracellular protein and the binding partner may be the entity normally bound *in vivo*. In other embodiments, however, the binding partner may be the protein or peptide on which the protein-capture agent was selected (through *in vitro* or *in vivo* selection) or raised (as in the case of antibodies). A binding partner may be shared by more than one protein-capture agent. For instance, a binding partner which is bound by a variety of polyclonal antibodies may bear a number of different epitopes. One protein-capture agent may also bind to a multitude of binding partners, for instance, if the binding partners share the same epitope.

A "protein" means a polymer of amino acid residues linked together by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. Typically, however, a protein will be at least six

amino acids long. Preferably, if the protein is a short peptide, it will be at least about 10 amino acid residues long. A protein may be naturally occurring, recombinant, or synthetic, or any combination of these. A protein may also be just a fragment of a naturally occurring protein or peptide. A protein may be a single molecule or may be a multi-molecular complex. The term protein may also apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid. An amino acid polymer in which one or more amino acid residues is an "unnatural" amino acid, not corresponding to any naturally occurring amino acid, is also encompassed by the use of the term "protein" herein.

A "fragment of a protein" means a protein which is a portion of another protein. For instance, fragments of a proteins may be a polypeptides obtained by digesting full-length protein isolated from cultured cells. A fragment of a protein will typically comprise at least six amino acids. More typically, the fragment will comprise at least ten amino acids. Preferably, the fragment comprises at least about 16 amino acids.

An "expression product" is a biomolecule, such as a protein, which is produced when a gene in an organism is expressed. An expression product may optionally comprise post-translational modifications.

The term "antibody" means an immunoglobulin, whether natural or partially or wholly synthetically produced. All derivatives thereof which maintain specific binding ability are also included in the term. The term also covers any protein having a binding domain which is homologous or largely homologous to an immunoglobulin binding domain. These proteins may be derived from natural sources, or partly or wholly synthetically produced. An antibody may be monoclonal or polyclonal. The antibody may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgA, IgD, and IgE. Derivatives of the IgG class, however, are preferred in the present invention.

The term "antibody fragment" refers to any derivative of an antibody which is less than full-length. Preferably, the antibody fragment retains at least a significant portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')<sub>2</sub>, scFv, Fv, dsFv diabody, and Fd fragments. The antibody fragment may be produced by any means. For instance, the antibody fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or it may be recombinantly produced from a gene encoding the partial antibody sequence. Alternatively, the antibody fragment may be wholly or partially synthetically produced. The antibody fragment may optionally be a single chain antibody fragment. Alternatively, the fragment may comprise multiple chains which are linked together, for instance, by disulfide linkages. The fragment may also optionally be a multimolecular complex. A functional antibody fragment will typically comprise at least about 50 amino acids and more typically will comprise at least about 200 amino acids.

Single-chain Fvs (scFvs) are recombinant antibody fragments consisting of only the variable light chain (V<sub>L</sub>) and variable heavy chain (V<sub>H</sub>) covalently connected to one another by a polypeptide linker. Either V<sub>L</sub> or V<sub>H</sub> may be the NH<sub>2</sub>-terminal domain. The polypeptide linker may be of variable length and composition so long as the two variable domains are bridged without serious steric interference. Typically, the linkers are comprised primarily of stretches of glycine and serine residues with some glutamic acid or lysine residues interspersed for solubility.

"Diabodies" are dimeric scFvs. The components of diabodies typically have shorter peptide linkers than most scFvs and they show a preference for associating as dimers.

An "Fv" fragment consists of one V<sub>H</sub> and one V<sub>L</sub> domain held together by noncovalent interactions. The term "dsFv" is used herein to refer to an Fv with an engineered intermolecular disulfide bond to stabilize the V<sub>H</sub>-V<sub>L</sub> pair.

A "F(ab')<sub>2</sub>" fragment is an antibody fragment essentially equivalent to that obtained from immunoglobulins (typically IgG) by digestion with an enzyme pepsin at pH 4.0-4.5. The fragment may be recombinantly produced.

A "Fab'" fragment is an antibody fragment essentially equivalent to that obtained by reduction of the disulfide bridge or bridges joining the two heavy chain pieces in the F(ab')<sub>2</sub> fragment. The Fab' fragment may be recombinantly produced.

A "Fab" fragment is an antibody fragment essentially equivalent to that obtained by digestion of immunoglobulins (typically IgG) with the enzyme papain. The Fab fragment may be recombinantly produced. The heavy chain segment of the Fab fragment is the Fd piece.

A "population of cells in an organism" means a collection of more than one cell in a single organism or more than one cell originally derived from a single organism.

The cells in the collection are preferably all of the same type. They may all be from the same tissue in an organism, for instance. Most preferably, gene expression in all of the cells in the population is identical or nearly identical.

"Conditions suitable for protein binding" means those conditions (in terms of salt concentration, pH, detergent, protein concentration, temperature, etc.) which allow for binding to occur between an immobilized protein-capture agent and its binding partner in solution. Preferably, the conditions are not so lenient that a significant amount of nonspecific protein binding occurs.

A "body fluid" may be any liquid substance extracted, excreted, or secreted from an organism or tissue of an organism. The body fluid need not necessarily contain cells. Body fluids of relevance to the present invention include, but are not limited to, whole blood, serum, urine, plasma, cerebral spinal fluid, tears, sinovial fluid, and amniotic fluid.

An "array" is an arrangement of entities in a pattern on a substrate. Although the pattern is typically a two-dimensional pattern, the pattern may also be a three-dimensional pattern.

A "patch of protein-capture agents" means a discrete region of immobilized protein-capture agents on the surface of a substrate. The patches may be of any



geometric shape or may be irregularly shaped. For instance, the patch may be, but need not necessarily be, square in shape.

"Proteomics" means the study of or the characterization of either the proteome or some fraction of the proteome. The "proteome" is the total collection of the intracellular proteins of a cell or population of cells and the proteins secreted by the cell or population of cells. This characterization most typically includes measurements of the presence, and usually quantity, of the proteins which have been expressed by a cell. The function, structural characteristics (such as post translational modification), and location within the cell of the proteins may also be studied. "Functional proteomics" refers to the study of the functional characteristics, activity level, and structural characteristics of the protein expression products of a cell or population of cells.

The term "substrate" refers to the bulk, underlying, and core material of the arrays of the invention.

The terms "micromachining" and "microfabrication" both refer to any number of techniques which are useful in the generation of microstructures (structures with feature sizes of sub-millimeter scale). Such technologies include, but are not limited to, laser ablation, electrodeposition, physical and chemical vapor deposition, photolithography, and wet chemical and dry etching. Related technologies such as injection molding and LIGA (X-ray lithography, electrodeposition, and molding) are also included. Most of these techniques were originally developed for use in semiconductors, microelectronics, and Micro-ElectroMechanical Systems (MEMS) but are applicable to the present invention as well.

The term "coating" means a layer that is either naturally or synthetically formed on or applied to the surface of the substrate. For instance, exposure of a substrate, such as silicon, to air results in oxidation of the exposed surface. In the case of a substrate made of silicon, a silicon oxide coating is formed on the surface upon exposure to air. In other instances, the coating is not derived from the substrate and may be placed upon the surface via mechanical, physical, electrical, or

chemical means. An example of this type of coating would be a metal coating that is applied to a silicon or polymer substrate or a silicon nitride coating that is applied to a silicon substrate. Although a coating may be of any thickness, typically the coating has a thickness smaller than that of the substrate.

An "interlayer" is an additional coating or layer that is positioned between the first coating and the substrate. Multiple interlayers may optionally be used together.

The primary purpose of a typical interlayer is to aid adhesion between the first coating and the substrate. One such example is the use of a titanium or chromium interlayer to help adhere a gold coating to a silicon or glass surface. However, other possible functions of an interlayer are also anticipated. For instance, some interlayers may perform a role in the detection system of the array (such as a semiconductor or metal layer between a nonconductive substrate and a nonconductive coating).

An "organic thinfilm" is a thin layer of organic molecules which has been applied to a substrate or to a coating on a substrate if present. Typically, an organic thinfilm is less than about 20 nm thick. Optionally, an organic thinfilm may be less than about 10 nm thick. An organic thinfilm may be disordered or ordered. For instance, an organic thinfilm can be amorphous (such as a chemisorbed or spin-coated polymer) or highly organized (such as a Langmuir-Blodgett film or self-assembled monolayer). An organic thinfilm may be heterogeneous or homogeneous. Organic thinfilms which are monolayers are preferred. A lipid bilayer or monolayer is a preferred organic thinfilm. Optionally, the organic thinfilm may comprise a combination of more than one form of organic thinfilm. For instance, an organic thinfilm may comprise a lipid bilayer on top of a self-assembled monolayer. A hydrogel may also compose an organic thinfilm. The organic thinfilm will typically have functionalities exposed on its surface which serve to enhance the surface conditions of a substrate or the coating on a substrate in any of a number of ways. For instance, exposed functionalities of the organic thinfilm are typically useful in the binding or covalent immobilization of the protein-capture agents to the patches of the array. Alternatively, the organic

thinfilm may bear functional groups (such as polyethylene glycol (PEG)) which reduce the non-specific binding of molecules to the surface. Other exposed functionalities serve to tether the thinfilm to the surface of the substrate or the coating. Particular functionalities of the organic thinfilm may also be designed to enable certain detection techniques to be used with the surface. Alternatively, the organic thinfilm may serve the purpose of preventing inactivation of a protein-capture agent or the protein to be bound by a protein-capture agent from occurring upon contact with the surface of a substrate or a coating on the surface of a substrate.

A "monolayer" is a single-molecule thick organic thinfilm. A monolayer may be disordered or ordered. A monolayer may optionally be a polymeric compound, such as a polyanionic polymer, a polyionic polymer, or a block-copolymer. For instance, the monolayer may be composed of a poly(amino acid) such as polylysine. A monolayer which is a self-assembled monolayer, however, is most preferred. One face of the self-assembled monolayer is typically composed of chemical functionalities on the termini of the organic molecules that are chemisorbed or physisorbed onto the surface of the substrate or, if present, the coating on the substrate if present. Examples of suitable functionalities of monolayers include the positively charged amino groups of poly-L-lysine for use on negatively charged surfaces and thiols for use on gold surfaces. Typically, the other face of the self-assembled monolayer is exposed and may bear any number of chemical functionalities (end groups). Preferably, the molecules of the self-assembled monolayer are highly ordered.

A "self-assembled monolayer" is a monolayer which is created by the spontaneous assembly of molecules. The self-assembled monolayer may be ordered, disordered, or exhibit short- to long-range order.

An "affinity tag" is a functional moiety capable of directly or indirectly immobilizing a protein-capture agent onto an exposed functionality of the organic thinfilm. Preferably, the affinity tag enables the site-specific immobilization and thus enhances orientation of the protein-capture agent onto the organic thinfilm.

In some cases, the affinity tag may be a simple chemical functional group. Other possibilities include amino acids, poly(amino acid) tags, or full-length proteins. Still other possibilities include carbohydrates and nucleic acids. For instance, the affinity tag may be a polynucleotide which hybridizes to another polynucleotide serving as a functional group on the organic thinfilm or another polynucleotide serving as an adaptor. The affinity tag may also be a synthetic chemical moiety. If the organic thinfilm of each of the patches comprises a lipid bilayer or monolayer, then a membrane anchor is a suitable affinity tag. The affinity tag may be covalently or noncovalently attached to the protein-capture agent. For instance, if the affinity tag is covalently attached to the protein-capture agent it may be attached via chemical conjugation or as a fusion protein. The affinity tag may also be attached to the protein-capture agent via a cleavable linkage.

Alternatively, the affinity tag may not be directly in contact with the protein-capture agent. The affinity tag may instead be separated from the protein-capture agent by an adaptor. The affinity tag may immobilize the protein-capture agent to the organic thinfilm either through noncovalent interactions or through a covalent linkage.

An "adaptor", for purposes of this invention, is any entity that links an affinity tag to the protein-capture agent. The adaptor may be, but need not necessarily be, a discrete molecule that is noncovalently attached to both the affinity tag and the protein-capture agent. The adaptor can instead be covalently attached to the affinity tag or the protein-capture agent or both (via chemical conjugation or as a fusion protein, for instance). Proteins such as full-length proteins, polypeptides, or peptides are typical adaptors. Other possible adaptors include carbohydrates or nucleic acids.

The term "fusion protein" refers to a protein composed of two or more polypeptides that, although typically unjoined in their native state, are joined by their respective amino and carboxyl termini through a peptide linkage to form a single continuous polypeptide. It is understood that the two or more polypeptide

components can either be directly joined or indirectly joined through a peptide linker/spacer.

The term "normal physiological condition" means conditions that are typical inside a living organism or a cell. While it is recognized that some organs or organisms provide extreme conditions, the intra-organismal and intra-cellular environment normally varies around pH 7 (*i.e.*, from pH 6.5 to pH 7.5), contains water as the predominant solvent, and exists at a temperature above 0°C and below 50°C. It will be recognized that the concentration of various salts depends on the organ, organism, cell, or cellular compartment used as a reference.

(b) Arrays of the invention.

The present invention is directed to arrays of protein-capture agents which can bind a plurality of proteins that are the expression products, or fragments thereof, of a cell or population of cells in an organism and therefore can be used to evaluate gene expression at the protein level. Typically, the arrays comprise micrometer-scale, two-dimensional patterns of patches of protein-capture agents immobilized on an organic thinfilm coating on the surface of the substrate.

In one embodiment of the invention, the array of protein-capture agents comprises a substrate, at least one organic thinfilm covering some or all of the surface of the substrate, and a plurality of patches arranged in discrete, known regions on the portions of the substrate surface covered by organic thinfilm, wherein (i) each patch comprises protein-capture agents immobilized on the organic thinfilm, wherein said protein-capture agents of a given patch are capable of binding a particular expression product, or a fragment thereof, of a cell or population of cells in an organism, and (ii) the array comprises a plurality of different protein-capture agents, each of which is capable of binding a different expression product, or fragment thereof, of the cell or population of cells.

The protein-capture agents are preferably covalently immobilized on the patches of the array, either directly or indirectly.

In most cases, the array will comprise at least about ten patches. In a preferred embodiment, the array comprises at least about 50 patches. In a particularly preferred embodiment the array comprises at least about 100 patches. In alternative preferred embodiments, the array of protein-capture agents may comprise more than  $10^3$ ,  $10^4$  or  $10^5$  patches.

The area of surface of the substrate covered by each of the patches is preferably no more than about  $0.25 \text{ mm}^2$ . Preferably, the area of the substrate surface covered by each of the patches is between about  $1 \mu\text{m}^2$  and about  $10,000 \mu\text{m}^2$ . In a particularly preferred embodiment, each patch covers an area of the substrate surface from about  $100 \mu\text{m}^2$  to about  $2,500 \mu\text{m}^2$ . In an alternative embodiment, a patch on the array may cover an area of the substrate surface as small as about  $2,500 \text{ nm}^2$ , although patches of such small size are generally not necessary for the use of the array.

The patches of the array may be of any geometric shape. For instance, the patches may be rectangular or circular. The patches of the array may also be irregularly shaped. The patches are optionally elevated from the median plan of the underlying substrate.

The distance separating the patches of the array can vary. Preferably, the patches of the array are separated from neighboring patches by about  $1 \mu\text{m}$  to about  $500 \mu\text{m}$ . Typically, the distance separating the patches is roughly proportional to the diameter or side length of the patches on the array if the patches have dimensions greater than about  $10 \mu\text{m}$ . If the patch size is smaller, then the distance separating the patches will typically be larger than the dimensions of the patch.

In a preferred embodiment of the array, the patches of the array are all contained within an area of about  $1 \text{ cm}^2$  or less on the surface of the substrate. In one preferred embodiment of the array, therefore, the array comprises 100 or more patches within a total area of about  $1 \text{ cm}^2$  or less on the surface of the substrate. Alternatively, a particularly preferred array comprises  $10^3$  or more patches within a total area of about  $1 \text{ cm}^2$  or less. A preferred array may even optionally comprise  $10^4$  or  $10^5$  or more patches within an area of about  $1 \text{ cm}^2$  or less on the

surface of the substrate. In other embodiments of the invention, all of the patches of the array are contained within an area of about 1 mm<sup>2</sup> or less on the surface of the substrate.

Typically, only one type of protein-capture agent is present on a single patch of the array. If more than one type of protein-capture agent is present on a single patch, all of the protein-capture agents of that patch must share a common binding partner. For instance, a patch may comprise a variety of polyclonal antibodies to the same antigen (although, potentially, the antibodies may bind different epitopes on that same antigen).

The arrays of the invention can have any number of a plurality of different protein-capture agents. Typically the array comprises at least about ten different protein-capture agents. Preferably, the array comprises at least about 50 different protein-capture agents. More preferably, the array comprises at least about 100 different protein-capture agents. Alternative preferred arrays comprise more than about 10<sup>3</sup> different protein-capture agents or more than about 10<sup>4</sup> different protein-capture agents. The array may even optionally comprise more than about 10<sup>5</sup> different protein-capture agents.

The number of different protein-capture agents on the array will vary depending on the application desired. For instance, if the array is to be used as a diagnostic tool in evaluating the status of a tumor or other diseased tissue in a patient, an array comprising less than about 100 different protein-capture agents may suffice since the necessary binding partners of the protein-capture agent on the array are limited to only those proteins whose expression is known to be indicative of the disease condition. However, if the array is to be used to measure a significant portion of the total protein content of a cell, then the array preferably comprises at least about 10,000 different protein-capture agents. Alternatively, a more limited proteomics study, such as a study of the abundances of various human transcription factors, for instance, might only require an array of about 100 different protein-capture agents.

In one embodiment of the array, each of the patches of the array comprises a different protein-capture agent. For instance, an array comprising about 100 patches could comprise about 100 different protein-capture agents. Likewise, an array of about 10,000 patches could comprise about 10,000 different protein-capture agents. In an alternative embodiment, however, each different protein-capture agent is immobilized on more than one separate patch on the array. For instance, each different protein-capture agent may optionally be present on two to six different patches. An array of the invention, therefore, may comprise about three-thousand protein-capture agent patches, but only comprise about one thousand different protein-capture agents since each different protein-capture agent is present on three different patches.

Typically, the number of different proteins which can be bound by the plurality of different protein-capture agents on the array will be at least about ten. However, it is preferred that the plurality of different protein-capture agents on the array is capable of binding a higher number of different proteins, such as at least about 50 or at least about 100. In still further preferred embodiments, the plurality of different proteins on the array is capable of binding at least about  $10^3$  proteins. For some applications, such as those where it is desirable to assay the entire protein content of a cell, or a significant fraction thereof, an array where the plurality of protein-capture agents is capable of binding at least about  $10^4$  different proteins or even at least about  $10^5$  different proteins is most preferred.

In one embodiment of the invention, the binding partners of the plurality of protein-capture agents on the array are proteins which are all expression products, or fragments thereof, of a cell or population of cells of a single organism. The expression products may be proteins, including peptides, of any size or function. They may be intracellular proteins or extracellular proteins. The expression products may be from a one-celled or multicellular organism. The organism may be a plant or an animal. In a preferred embodiment of the invention, the binding partners are human expression products, or fragments thereof.



In one embodiment of the invention, the binding partners of the protein-capture agents of the array may be a randomly chosen subset of all the proteins, including peptides, which are expressed by a cell or population of cells in a given organism or a subset of all the fragments of those proteins. Thus, the binding partners of the protein-capture agents of the array optionally represent a wide distribution of different proteins from a single organism.

The binding partners of some or all of the protein-capture agents on the array need not necessarily be known. The binding partner of a protein-capture agent of the array may be a protein or peptide of unknown function. For instance, the different protein-capture agents of the array may together bind a wide range of cellular proteins from a single cell type, many of which are of unknown identity and/or function.

In another embodiment of the present invention, the binding partners of the protein-capture agents on the array are related proteins. The different proteins bound by the protein-capture agents may optionally be members of the same protein family. The different binding partners of the protein-capture agents of the array may be either functionally related or just suspected of being functionally related. The different proteins bound by the protein-capture agents of the array may also be proteins which share a similarity in structure or sequence or are simply suspected of sharing a similarity in structure or sequence. For instance, the binding partners of the protein-capture agents on the array may optionally all be growth factor receptors, hormone receptors, neurotransmitter receptors, catecholamine receptors, amino acid derivative receptors, cytokine receptors, extracellular matrix receptors, antibodies, lectins, cytokines, serpins, proteases, kinases, phosphatases, ras-like GTPases, hydrolases, steroid hormone receptors, transcription factors, heat-shock transcription factors, DNA-binding proteins, zinc-finger proteins, leucine-zipper proteins, homeodomain proteins, intracellular signal transduction modulators and effectors, apoptosis-related factors, DNA synthesis factors, DNA repair factors, DNA recombination factors, cell-surface antigens, hepatitis C virus (HCV) proteases or HIV proteases.

In an alternative embodiment of the invention, the proteins which are the binding partners of the protein-capture agents of the array may be fragments of the expression products of a cell or population of cells in an organism.

A protein-capture agent on the array can be any molecule or complex of molecules which has the ability to bind a protein and immobilize it to the site of the protein-capture agent on the array. Preferably, the protein-capture agent binds its binding partner in a substantially specific manner. Hence, the protein-capture agent may optionally be a protein whose natural function in a cell is to specifically bind another protein, such as an antibody or a receptor. Alternatively, the protein-capture agent may instead be a partially or wholly synthetic or recombinant protein which specifically binds a protein. Alternatively, the protein-capture agent may be a protein which has been selected *in vitro* from a mutagenized, randomized, or completely random and synthetic library by its binding affinity to a specific protein or peptide target. The selection method used may optionally have been a display method such as ribosome display or phage display (see below). Alternatively, the protein-capture agent obtained via *in vitro* selection may be a DNA or RNA aptamer which specifically binds a protein target (for example: Potyrailo *et al.*, *Anal. Chem.*, **70**:3419-25, 1998; Cohen, *et al.*, *Proc. Natl. Acad. Sci. USA*, **95**:14272-7, 1998; Fukuda, *et al.*, *Nucleic Acids Symp. Ser.*, (37):237-8, 1997). Alternatively, the *in vitro* selected protein-capture agent may be a polypeptide (Roberts and Szostak, *Proc. Natl. Acad. Sci. USA*, **94**:12297-302, 1997). In an alternative embodiment, the protein-capture agent may be a small molecule which has been selected from a combinatorial chemistry library or is isolated from an organism.

In a preferred embodiment of the array, however, the protein-capture agents are proteins. In a particularly preferred embodiment, the protein-capture agents are antibodies or antibody fragments. Although antibody moieties are exemplified herein, it is understood that the present arrays and methods may be advantageously employed with other protein-capture agents.

The antibodies or antibody fragments of the array may optionally be single-chain Fvs, Fab fragments, Fab' fragments, F(ab')<sub>2</sub> fragments, Fv fragments, dsFvs, diabodies, Fd fragments, full-length, antigen-specific polyclonal antibodies, or full-length monoclonal antibodies. In a preferred embodiment, the protein-capture agents of the array are monoclonal antibodies, Fab fragments or single-chain Fvs. The antibodies or antibody fragments may be monoclonal antibodies, even commercially available antibodies, against known, well-characterized proteins. Alternatively, the antibody fragments have been derived by selection from a library using the phage display method. If the antibody fragments are derived individually by selection based on binding affinity to known proteins, then, the binding partners of the antibody fragments are known. In an alternative embodiment of the invention, the antibody fragments have been derived by a phage display method comprising selection based on binding affinity to the (typically, immobilized) proteins of a cellular extract or a body fluid. In this embodiment, some or many of the antibody fragments of the array would bind proteins of unknown identity and/or function.

Upon using the array of protein-capture agents to bind a plurality of expression products, or fragments thereof, an array of bound proteins is created. Thus, another embodiment of the invention provides an array of bound proteins which comprises (a) a protein-capture agent array of the invention and (b) a plurality of different proteins which are expression products, or fragments thereof, of a cell or a population of cells in an organism, wherein each of the different proteins is bound to a protein-capture agent on a separate patch of the array. Preferably, each of the different proteins is non-covalently bound to a protein-capture agent.

(c) Substrates, coatings, and organic thinfilms.

The substrate of the array may be either organic or inorganic, biological or non-biological, or any combination of these materials. In one embodiment, the substrate is transparent or translucent. The portion of the surface of the substrate on which the patches reside is preferably flat and firm or semi-firm. However, the

array of the present invention need not necessarily be flat or entirely two-dimensional. Significant topological features may be present on the surface of the substrate surrounding the patches, between the patches or beneath the patches. For instance, walls or other barriers may separate the patches of the array. Numerous materials are suitable for use as a substrate in the array embodiment of the invention. For instance, the substrate of the invention array can comprise a material selected from a group consisting of silicon, silica, quartz, glass, controlled pore glass, carbon, alumina, titania, tantalum oxide, germanium, silicon nitride, zeolites, and gallium arsenide. Many metals such as gold, platinum, aluminum, copper, titanium, and their alloys are also options for substrates of the array. In addition, many ceramics and polymers may also be used as substrates. Polymers which may be used as substrates include, but are not limited to, the following: polystyrene; poly(tetra)fluoroethylene (PTFE); polyvinylidenedifluoride; polycarbonate; polymethylmethacrylate; polyvinylethylene; polyethyleneimine; poly(etherether)ketone; polyoxymethylene (POM); polyvinylphenol; polylactides; polymethacrylimide (PMI); polyalkenesulfone (PAS); polypropylethylene, polyethylene; polyhydroxyethylmethacrylate (HEMA); polydimethylsiloxane; polyacrylamide; polyimide; and block-copolymers. Preferred substrates for the array include silicon, silica, glass, and polymers. The substrate on which the patches reside may also be a combination of any of the aforementioned substrate materials. An array of the present invention may optionally further comprise a coating between the substrate and the organic thinfilm of its patches. This coating may either be formed on the substrate or applied to the substrate. The substrate can be modified with a coating by using thin-film technology based, for instance, on physical vapor deposition (PVD), plasma-enhanced chemical vapor deposition (PECVD), or thermal processing. Alternatively, plasma exposure can be used to directly activate or alter the substrate and create a coating. For instance, plasma etch procedures can be used to oxidize a polymeric surface (for example,

polystyrene or polyethylene to expose polar functionalities such as hydroxyls, carboxylic acids, aldehydes and the like) which then acts as a coating.

The coating is optionally a metal film. Possible metal films include aluminum, chromium, titanium, tantalum, nickel, stainless steel, zinc, lead, iron, copper, magnesium, manganese, cadmium, tungsten, cobalt, and alloys or oxides thereof. In a preferred embodiment, the metal film is a noble metal film. Noble metals that may be used for a coating include, but are not limited to, gold, platinum, silver, and copper. In an especially preferred embodiment, the coating comprises gold or a gold alloy. Electron-beam evaporation may be used to provide a thin coating of gold on the surface of the substrate. In a preferred embodiment, the metal film is from about 50 nm to about 500 nm in thickness. In an alternative embodiment, the metal film is from about 1 nm to about 1  $\mu\text{m}$  in thickness.

In alternative embodiments, the coating comprises a composition selected from the group consisting of silicon, silicon oxide, titania, tantalum oxide, silicon nitride, silicon hydride, indium tin oxide, magnesium oxide, alumina, glass, hydroxylated surfaces, and polymers.

In one embodiment of the invention array, the surface of the coating is atomically flat. In this embodiment, the mean roughness of the surface of the coating is less than about 5 angstroms for areas of at least 25  $\mu\text{m}^2$ . In a preferred embodiment, the mean roughness of the surface of the coating is less than about 3 angstroms for areas of at least 25  $\mu\text{m}^2$ . The ultraflat coating can optionally be a template-stripped surface as described in Hegner *et al.*, *Surface Science*, 1993, 291:39-46 and Wagner *et al.*, *Langmuir*, 1995, 11:3867-3875, both of which are incorporated herein by reference.

It is contemplated that the coatings of many arrays will require the addition of at least one adhesion layer between said coating and the substrate. Typically, the adhesion layer will be at least 6 angstroms thick and may be much thicker. For instance, a layer of titanium or chromium may be desirable between a silicon wafer and a gold coating. In an alternative embodiment, an epoxy glue such as Epo-tek 377®, Epo-tek 301-2®, (Epoxy Technology Inc., Billerica,

Massachusetts) may be preferred to aid adherence of the coating to the substrate. Determinations as to what material should be used for the adhesion layer would be obvious to one skilled in the art once materials are chosen for both the substrate and coating. In other embodiments, additional adhesion mediators or interlayers may be necessary to improve the optical properties of the array, for instance, waveguides for detection purposes.

Deposition or formation of the coating (if present) on the substrate is performed prior to the formation of the organic thinfilm thereon. Several different types of coating may be combined on the surface. The coating may cover the whole surface of the substrate or only parts of it. The pattern of the coating may or may not be identical to the pattern of organic thinfilms used to immobilize the protein-capture agents. In one embodiment of the invention, the coating covers the substrate surface only at the site of the patches of protein-capture agents.

Techniques useful for the formation of coated patches on the surface of the substrate which are organic thinfilm compatible are well known to those of ordinary skill in the art. For instance, the patches of coatings on the substrate may optionally be fabricated by photolithography, micromolding (PCT Publication WO 96/29629), wet chemical or dry etching, or any combination of these.

The organic thinfilm on which each of the patches of protein-capture agents resides forms a layer either on the substrate itself or on a coating covering the substrate. The organic thinfilm on which the protein-capture agents of the patches are immobilized is preferably less than about 20 nm thick. In some embodiments of the invention, the organic thinfilm of each of the patches may be less than about 10 nm thick.

A variety of different organic thinfilms are suitable for use in the present invention. Methods for the formation of organic thinfilms include *in situ* growth from the surface, deposition by physisorption, spin-coating, chemisorption, self-assembly, or plasma-initiated polymerization from gas phase. For instance, a hydrogel composed of a material such as dextran can serve as a suitable organic thinfilm on the patches of the array. In one preferred embodiment of the

invention, the organic thinfilm is a lipid bilayer. In another preferred embodiment, the organic thinfilm of each of the patches of the array is a monolayer. A monolayer of polyarginine or polylysine adsorbed on a negatively charged substrate or coating is one option for the organic thinfilm. Another option is a disordered monolayer of tethered polymer chains. In a particularly preferred embodiment, the organic thinfilm is a self-assembled monolayer. The organic thinfilm is most preferably a self-assembled monolayer which comprises molecules of the formula  $X-R-Y$ , wherein  $R$  is a spacer,  $X$  is a functional group that binds  $R$  to the surface, and  $Y$  is a functional group for binding protein-capture agents onto the monolayer. In an alternative preferred embodiment, the self-assembled monolayer is comprised of molecules of the formula  $(X)_aR(Y)_b$  where  $a$  and  $b$  are, independently, integers greater than or equal to 1 and  $X$ ,  $R$ , and  $Y$  are as previously defined. In an alternative preferred embodiment, the organic thinfilm comprises a combination of organic thinfilms such as a combination of a lipid bilayer immobilized on top of a self-assembled monolayer of molecules of the formula  $X-R-Y$ . As another example, a monolayer of polylysine can also optionally be combined with a self-assembled monolayer of molecules of the formula  $X-R-Y$  (see US Patent No. 5,629,213).

In all cases, the coating, or the substrate itself if no coating is present, must be compatible with the chemical or physical adsorption of the organic thinfilm on its surface. For instance, if the patches comprise a coating between the substrate and a monolayer of molecules of the formula  $X-R-Y$ , then it is understood that the coating must be composed of a material for which a suitable functional group  $X$  is available (see below). If no such coating is present, then it is understood that the substrate must be composed of a material for which a suitable functional group  $X$  is available.

In a preferred embodiment of the invention, the regions of the substrate surface, or coating surface, which separate the patches of protein-capture agents are free of organic thinfilm. In an alternative embodiment, the organic thinfilm extends beyond the area of the substrate surface, or coating surface if present, covered by

the patches of protein-capture agents. For instance, optionally, the entire surface of the array may be covered by an organic thinfilm on which the plurality of spatially distinct patches of protein-capture agents reside. An organic thinfilm which covers the entire surface of the array may be homogenous or may optionally comprise patches of differing exposed functionalities useful in the immobilization of patches of different protein-capture agents. In still another alternative embodiment, the regions of the substrate surface or coating surface, if a coating is present, between the patches of protein-capture agents are covered by an organic thinfilm, but an organic thinfilm of a different type than that of the patches of protein-capture agents. For instance, the surfaces between the patches of protein-capture agents may be coated with an organic thinfilm characterized by low non-specific binding properties for proteins and other analytes.

A variety of techniques may be used to generate patches of organic thinfilm on the surface of the substrate or on the surface of a coating on the substrate. These techniques are well known to those skilled in the art and will vary depending upon the nature of the organic thinfilm, the substrate, and the coating if present. The techniques will also vary depending on the structure of the underlying substrate and the pattern of any coating present on the substrate. For instance, patches of a coating which is highly reactive with an organic thinfilm may have already been produced on the substrate surface. Arrays of patches of organic thinfilm can optionally be created by microfluidics printing, microstamping (US Patent Nos. 5,512,131 and 5,731,152), or microcontact printing ( $\mu$ CP) (PCT Publication WO 96/29629). Subsequent immobilization of protein-capture agents to the reactive monolayer patches results in two-dimensional arrays of the agents. Inkjet printer heads provide another option for patterning monolayer X-R-Y molecules, or components thereof, or other organic thinfilm components to nanometer or micrometer scale sites on the surface of the substrate or coating (Lemmo *et al.*, *Anal Chem.*, 1997, 69:543-551; US Patent Nos. 5,843,767 and 5,837,860). In some cases, commercially available arrayers based on capillary dispensing (for instance, OmniGrid™ from Genemachines, inc, San Carlos, CA, and High-



Throughput Microarrayer from Intelligent Bio-Instruments, Cambridge, MA) may also be of use in directing components of organic thinfilms to spatially distinct regions of the array.

Diffusion boundaries between the patches of protein-capture agents immobilized on organic thinfilms such as self-assembled monolayers may be integrated as topographic patterns (physical barriers) or surface functionalities with orthogonal wetting behavior (chemical barriers). For instance, walls of substrate material or photoresist may be used to separate some of the patches from some of the others or all of the patches from each other. Alternatively, non-bioreactive organic thinfilms, such as monolayers, with different wettability may be used to separate patches from one another.

In a preferred embodiment of the invention, each of the patches of protein-capture agents comprises a self-assembled monolayer of molecules of the formula X-R-Y, as previously defined, and the patches are separated from each other by surfaces free of the monolayer.

Figure 1 shows the top view of one example of an array of patches reactive with protein-capture agents. On the array, a number of patches 15 cover the surface of the substrate 3.

Figure 2 shows a detailed cross section of a patch 15 of the array of Figure 1.

This view illustrates the use of a coating 5 on the substrate 3. An adhesion interlayer 6 is also included in the patch. On top of the patch resides a self-assembled monolayer 7.

Figure 3 shows a cross section of one row of the patches 15 of the array of Figure 1. This figure also shows the use of a cover 2 over the array. Use of the cover 2 creates an inlet port 16 and an outlet port 17 for solutions to be passed over the array.

A variety of chemical moieties may function as monolayer molecules of the formula X-R-Y in the array of the present invention. However, three major classes of monolayer formation are preferably used to expose high densities of reactive omega-functionalities on the patches of the array: (i) alkylsiloxane

monolayers ("silanes") on hydroxylated and non-hydroxylated surfaces (as taught in, for example, US Patent No. 5,405,766, PCT Publication WO 96/38726, US Patent No. 5,412,087, and US Patent No. 5,688,642); (ii) alkyl-thiol/dialkyldisulfide monolayers on noble metals (preferably Au(111)) (as, for example, described in Allara *et al.*, US 4,690,715; Bamdad *et al.*, US 5,620,850; Wagner *et al.*, *Biophysical Journal*, 1996, 70:2052-2066); and (iii) alkyl monolayer formation on oxide-free passivated silicon (as taught in, for example, Linford *et al.*, *J. Am. Chem. Soc.*, 1995, 117:3145-3155, Wagner *et al.*, *Journal of Structural Biology*, 1997, 119:189-201, US Patent No. 5,429,708). One of ordinary skill in the art, however, will recognize that many possible moieties may be substituted for X, R, and/or Y, dependent primarily upon the choice of substrate, coating, and affinity tag. Many examples of monolayers are described in Ulman, *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self Assembly*, Academic press (1991).

In one embodiment, the monolayer comprises molecules of the formula  $(X)_aR(Y)_b$  wherein a and b are, independently, equal to an integer between 1 and about 200. In a preferred embodiment, a and b are, independently, equal to an integer between 1 and about 80. In a more preferred embodiment, a and b are, independently, equal to 1 or 2. In a most preferred embodiment, a and b are both equal to 1 (molecules of the formula X-R-Y).

If the patches of the invention array comprise a self-assembled monolayer of molecules of the formula  $(X)_aR(Y)_b$ , then R may optionally comprise a linear or branched hydrocarbon chain from about 1 to about 400 carbons long. The hydrocarbon chain may comprise an alkyl, aryl, alkenyl, alkynyl, cycloalkyl, alkaryl, aralkyl group, or any combination thereof. If a and b are both equal to one, then R is typically an alkyl chain from about 3 to about 30 carbons long. In a preferred embodiment, if a and b are both equal to one, then R is an alkyl chain from about 8 to about 22 carbons long and is, optionally, a straight alkane.

However, it is also contemplated that in an alternative embodiment, R may readily comprise a linear or branched hydrocarbon chain from about 2 to about 400

carbons long and be interrupted by at least one hetero atom. The interrupting hetero groups can include -O-, -CONH-, -CONHCO-, -NH-, -CSNH-, -CO-, -CS-, -S-, -SO-,  $-(\text{OCH}_2\text{CH}_2)_n$ - (where  $n=1-20$ ),  $-(\text{CF}_2)_n$ - (where  $n=1-22$ ), and the like. Alternatively, one or more of the hydrogen moieties of R can be substituted with deuterium. In alternative, less preferred, embodiments, R may be more than about 400 carbons long.

X may be chosen as any group which affords chemisorption or physisorption of the monolayer onto the surface of the substrate (or the coating, if present). When the substrate or coating is a metal or metal alloy, X, at least prior to incorporation into the monolayer, can in one embodiment be chosen to be an asymmetrical or symmetrical disulfide, sulfide, diselenide, selenide, thiol, isonitrile, selenol, a trivalent phosphorus compound, isothiocyanate, isocyanate, xanthanate, thiocarbamate, a phosphine, an amine, thio acid or a dithio acid. This embodiment is especially preferred when a coating or substrate is used that is a noble metal such as gold, silver, or platinum.

If the substrate of the array is a material such as silicon, silicon oxide, indium tin oxide, magnesium oxide, alumina, quartz, glass, or silica, then the array of one embodiment of the invention comprises an X that, prior to incorporation into said monolayer, is a monohalosilane, dihalosilane, trihalosilane, trialkoxysilane, dialkoxysilane, or a monoalkoxysilane. Among these silanes, trichlorosilane and trialkoxysilane are particularly preferred.

In a preferred embodiment of the invention, the substrate is selected from the group consisting of silicon, silicon dioxide, indium tin oxide, alumina, glass, and titania; and X, prior to incorporation into said monolayer, is selected from the group consisting of a monohalosilane, dihalosilane, trihalosilane, trichlorosilane, trialkoxysilane, dialkoxysilane, monoalkoxysilane, carboxylic acids, and phosphates.

In another preferred embodiment of the invention, the substrate of the array is silicon and X is an olefin.

In still another preferred embodiment of the invention, the coating (or the substrate if no coating is present) is titania or tantalum oxide and X is a phosphate. In other embodiments, the surface of the substrate (or coating thereon) is composed of a material such as titanium oxide, tantalum oxide, indium tin oxide, magnesium oxide, or alumina where X is a carboxylic acid or alkylphosphoric acid. Alternatively, if the surface of the substrate (or coating thereon) of the array is copper, then X may optionally be a hydroxamic acid.

If the substrate used in the invention is a polymer, then in many cases a coating on the substrate such as a copper coating will be included in the array. An appropriate functional group X for the coating would then be chosen for use in the array. In an alternative embodiment comprising a polymer substrate, the surface of the polymer may be plasma-modified to expose desirable surface functionalities for monolayer formation. For instance, EP 780423 describes the use of a monolayer molecule that has an alkene X functionality on a plasma exposed surface. Still another possibility for the invention array comprised of a polymer is that the surface of the polymer on which the monolayer is formed is functionalized by copolymerization of appropriately functionalized precursor molecules.

Another possibility is that prior to incorporation into the monolayer, X can be a free-radical-producing moiety. This functional group is especially appropriate when the surface on which the monolayer is formed is a hydrogenated silicon surface. Possible free-radical producing moieties include, but are not limited to, diacylperoxides, peroxides, and azo compounds. Alternatively, unsaturated moieties such as unsubstituted alkenes, alkynes, cyano compounds and isonitrile compounds can be used for X, if the reaction with X is accompanied by ultraviolet, infrared, visible, or microwave radiation.

In alternative embodiments, X, prior to incorporation into the monolayer, may be a hydroxyl, carboxyl, vinyl, sulfonyl, phosphoryl, silicon hydride, or an amino group.

The component, Y, of the monolayer is a functional group responsible for binding a protein-capture agent onto the monolayer. In a preferred embodiment of the invention, the Y group is either highly reactive (activated) towards the protein-capture agent (or its affinity tag) or is easily converted into such an activated form. In a preferred embodiment, the coupling of Y with the protein-capture agent occurs readily under normal physiological conditions not detrimental to the ability of the protein-capture agent to bind its binding partner. The functional group Y may either form a covalent linkage or a noncovalent linkage with the protein-capture agent (or its affinity tag, if present). In a preferred embodiment, the functional group Y forms a covalent linkage with the protein-capture agent or its affinity tag. It is understood that following the attachment of the protein-capture agent (with or without an affinity tag) to Y, the chemical nature of Y may have changed. Upon attachment of the protein-capture agent, Y may even have been removed from the organic thinfilm.

In one embodiment of the array of the present invention, Y is a functional group that is activated in situ. Possibilities for this type of functional group include, but are not limited to, such simple moieties such as a hydroxyl, carboxyl, amino, aldehyde, carbonyl, methyl, methylene, alkene, alkyne, carbonate, aryl iodide, or a vinyl group. Appropriate modes of activation would be obvious to one skilled in the art. Alternatively, Y can comprise a functional group that requires photoactivation prior to becoming activated enough to trap the protein-capture agent.

In an especially preferred embodiment of the array of the present invention, Y is a complex and highly reactive functional moiety that is compatible with monolayer formation and needs no *in situ* activation prior to reaction with the protein-capture agent and/or affinity tag. Such possibilities for Y include, but are not limited to, maleimide, N-hydroxysuccinimide (Wagner *et al.*, *Biophysical Journal*, 1996, 70:2052-2066), nitrilotriacetic acid (US Patent No. 5,620,850), activated hydroxyl, haloacetyl, bromoacetyl, iodoacetyl, activated carboxyl, hydrazide, epoxy, aziridine, sulfonylchloride, trifluoromethyldiaziridine, pyridyldisulfide, N-

acyl-imidazole, imidazolecarbamate, vinylsulfone, succinimidylcarbonate, arylazide, anhydride, diazoacetate, benzophenone, isothiocyanate, isocyanate, imidoester, fluorobenzene, and biotin.

Figure 4 shows one example of a monolayer on a substrate 3. In this example, substrate 3 comprises glass. The monolayer is thiolreactive because it bears a maleimidyl functional group Y.

Figure 5 shows another example of a monolayer on a substrate 3 which is silicon. In this case, however, a thinfilm gold coating 5 covers the surface of the substrate 3. Also, in this embodiment, a titanium adhesion interlayer 6 is used to adhere the coating 5 to the substrate 3. This monolayer is aminoreactive because it bears an N-hydroxysuccinimidyl functional group Y.

In an alternative embodiment, the functional group Y of the array is selected from the group of simple functional moieties. Possible Y functional groups include, but are not limited to, -OH, -NH<sub>2</sub>, -COOH, -COOR, -RSR, -PO<sub>4</sub><sup>-3</sup>, -OSO<sub>3</sub><sup>-2</sup>, -SO<sub>3</sub><sup>-</sup>, -COO<sup>-</sup>, -SOO<sup>-</sup>, -CONR<sub>2</sub>, -CN, -NR<sub>2</sub>, and the like.

The monolayer molecules of the present invention can optionally be assembled on the surface in parts. In other words, the monolayer need not necessarily be constructed by chemisorption or physisorption of molecules of the formula X-R-Y to the surface of the substrate (or coating). Instead, in one embodiment, X may be chemisorbed or physisorbed to the surface of the substrate (or coating) alone first. Then, R or even just individual components of R can be attached to X through a suitable chemical reaction. Upon completion of addition of the spacer R to the X moiety already immobilized on the surface, Y can be attached to the ends of the monolayer molecule through a suitable covalent linkage.

Not all self-assembled monolayer molecules on a given patch need be identical to one another. Some patches may comprise mixed monolayers. For instance, the monolayer of an individual patch may optionally comprise at least two different molecules of the formula X-R-Y, as previously described. This second X-R-Y molecule may immobilize the same or a different protein-capture agent having the

same binding partner as the first. In addition, some of the monolayer molecules X-R-Y of a patch may have failed to attach any protein-capture agent.

As another alternative embodiment of the invention, a mixed, self-assembled monolayer of an individual patch on the array may comprise both molecules of the formula X-R-Y, as previously described, and molecules of the formula, X-R-V where R is a spacer, X is a functional group that binds R to the surface, and V is a moiety which is biocompatible with proteins and resistant to the non-specific binding of proteins. For example, V may consist of a hydroxyl, saccharide, or oligo/polyethylene glycol moiety (EP Publication 780423).

In still another embodiment of the invention, the array comprises at least one unreactive patch of organic thinfilm on the substrate or coating surface which is devoid of any protein-capture agent. For instance, the unreactive patch may optionally comprise a monolayer of molecules of the formula X-R-V, where R is a spacer, X is a functional group that binds R to the surface, and V is a moiety resistant to the non-specific binding of proteins. The unreactive patch may serve as a control patch or be useful in background binding measurements.

Regardless of the nature of the monolayer molecules, in some arrays it may be desirable to provide crosslinking between molecules of an individual patch's monolayer. In general, crosslinking confers additional stability to the monolayer. Such methods are familiar to those skilled in the art (for instance, see Ulman, *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self-Assembly*, Academic Press (1991)).

After completion of formation of the monolayer on the patches, the protein-capture agent may be attached to the monolayer via interaction with the Y-functional group. Y-functional groups which fail to react with any protein-capture agents are preferably quenched prior to use of the array.

(d) Affinity tags and immobilization of protein-capture agents.

In a preferred embodiment, the protein-immobilizing patches of the array further comprise an affinity tag that enhances immobilization of the protein-capture agent

onto the organic thinfilm. The use of an affinity tag on the protein-capture agent of the array typically provides several advantages. An affinity tag can confer enhanced binding or reaction of the protein-capture agent with the functionalities on the organic thinfilm, such as Y if the organic thinfilm is a an X-R-Y monolayer as previously described. This enhancement effect may be either kinetic or thermodynamic. The affinity tag/thinfilm combination used in the patches of the array preferably allows for immobilization of the protein-capture agents in a manner which does not require harsh reaction conditions that are adverse to protein stability or function. In most embodiments, immobilization to the organic thinfilm in aqueous, biological buffers is ideal.

An affinity tag also preferably offers immobilization on the organic thinfilm that is specific to a designated site or location on the protein-capture agent (site-specific immobilization). For this to occur, attachment of the affinity tag to the protein-capture agent must be site-specific. Site-specific immobilization helps ensure that the protein-binding site of the agent, such as the antigen-binding site of the antibody moiety, remains accessible to ligands in solution. Another advantage of immobilization through affinity tags is that it allows for a common immobilization strategy to be used with multiple, different protein-capture agents.

The affinity tag is optionally attached directly, either covalently or noncovalently, to the protein-capture agent. In an alternative embodiment, however, the affinity tag is either covalently or noncovalently attached to an adaptor which is either covalently or noncovalently attached to the protein-capture agent.

In a preferred embodiment, the affinity tag comprises at least one amino acid. The affinity tag may be a polypeptide comprising at least two amino acids which is reactive with the functionalities of the organic thinfilm. Alternatively, the affinity tag may be a single amino acid which is reactive with the organic thinfilm.

Examples of possible amino acids which could be reactive with an organic thinfilm include cysteine, lysine, histidine, arginine, tyrosine, aspartic acid, glutamic acid, tryptophan, serine, threonine, and glutamine. A polypeptide or amino acid affinity tag is preferably expressed as a fusion protein with the protein-



capture agent when the protein-capture agent is a protein, such as an antibody or antibody fragment. Amino acid affinity tags provide either a single amino acid or a series of amino acids that can interact with the functionality of the organic thinfilm, such as the Y-functional group of the self-assembled monolayer molecules. Amino acid affinity tags can be readily introduced into recombinant proteins to facilitate oriented immobilization by covalent binding to the Y-functional group of a monolayer or to a functional group on an alternative organic thinfilm.

The affinity tag may optionally comprise a poly(amino acid) tag. A poly(amino acid) tag is a polypeptide that comprises from about 2 to about 100 residues of a single amino acid, optionally interrupted by residues of other amino acids. For instance, the affinity tag may comprise a poly-cysteine, polylysine, poly-arginine, or poly-histidine. Amino acid tags are preferably composed of two to twenty residues of a single amino acid, such as, for example, histidines, lysines, arginines, cysteines, glutamines, tyrosines, or any combination of these.

According to a preferred embodiment, an amino acid tag of one to twenty amino acids includes at least one to ten cysteines for thioether linkage; or one to ten lysines for amide linkage; or one to ten arginines for coupling to vicinal dicarbonyl groups. One of ordinary skill in the art can readily pair suitable affinity tags with a given functionality on an organic thinfilm.

The position of the amino acid tag can be at an amino-, or carboxy-terminus of the protein-capture agent which is a protein, or anywhere in-between, as long as the protein-binding region of the protein-capture agent, such as the antigen-binding region of an immobilized antibody moiety, remains in a position accessible for protein binding. Where compatible with the protein-capture agent chosen, affinity tags introduced for protein purification are preferentially located at the C-terminus of the recombinant protein to ensure that only full-length proteins are isolated during protein purification. For instance, if intact antibodies are used on the arrays, then the attachment point of the affinity tag on the antibody is preferably located at a C-terminus of the effector (Fc) region of the antibody. If scFvs are

used on the arrays, then the attachment point of the affinity tag is also preferably located at the C-terminus of the molecules.

Affinity tags may also contain one or more unnatural amino acids. Unnatural amino acids can be introduced using suppressor tRNAs that recognize stop codons (*i.e.*, amber) (Noren *et al.*, *Science*, 1989, 244:182-188; Ellman *et al.*, *Methods Enzym.*, 1991, 202:301-336; Cload *et al.*, *Chem. Biol.*, 1996, 3:1033-1038). The tRNAs are chemically amino-acylated to contain chemically altered ("unnatural") amino acids for use with specific coupling chemistries (*i.e.*, ketone modifications, photoreactive groups).

In an alternative embodiment the affinity tag can comprise an intact protein, such as, but not limited to, glutathione S-transferase, an antibody, avidin, or streptavidin.

When the protein-capture agent is a protein and the affinity tag is a protein, such as a poly(amino acid) tag, or a single amino acid, the affinity tag is preferably attached to the protein-capture agent by generating a fusion protein. Alternatively, protein synthesis or protein ligation techniques known to those skilled in the art may be used. For instance, intein-mediated protein ligation may optionally be used to attach the affinity tag to the protein-capture agent (Mathys, *et al.*, *Gene* 231:1-13, 1999; Evans, *et al.*, *Protein Science* 7:2256-2264, 1998).

Other protein conjugation and immobilization techniques known in the art may be adapted for the purpose of attaching affinity tags to the protein-capture agent. For instance, in an alternative embodiment of the array, the affinity tag may be an organic bioconjugate which is chemically coupled to the protein-capture agent of interest. Biotin or antigens may be chemically cross linked to the protein.

Alternatively, a chemical crosslinker may be used that attaches a simple functional moiety such as a thiol or an amine to the surface of a protein serving as a protein-capture agent on the array.

In an alternative embodiment of the invention, the organic thinfilm of each of the patches comprises, at least in part, a lipid monolayer or bilayer, and the affinity tag comprises a membrane anchor.

Figure 6 shows a detailed cross section of a patch on one embodiment of the invention array. In this embodiment, a protein-capture agent 10 is immobilized on a monolayer 7 on a substrate 3. An affinity tag 8 connects the protein-capture agent 10 to the monolayer 7. The monolayer 7 is formed on a coating 5 which is separated from the substrate 3 by an interlayer 6.

In an alternative embodiment of the invention, no affinity tag is used to immobilize the protein-capture agents onto the organic thinfilm. An amino acid or other moiety (such as a carbohydrate moiety) inherent to the protein-capture agent itself may instead be used to tether the protein-capture agent to the reactive group of the organic thinfilm. In preferred embodiments, the immobilization is site-specific with respect to the location of the site of immobilization on the protein-capture agent. For instance, the sulfhydryl group on the C-terminal region of the heavy chain portion of a Fab' fragment generated by pepsin digestion of an antibody, followed by selective reduction of the disulfide between monovalent Fab' fragments, may be used as the affinity tag. Alternatively, a carbohydrate moiety on the Fc portion of an intact antibody can be oxidized under mild conditions to an aldehyde group suitable for immobilizing the antibody on a monolayer via reaction with a hydrazide-activated Y group on the monolayer. Examples of immobilization of protein-capture agents without any affinity tag in a site-specific manner can be found in Dammer *et al.*, *Biophys J.*, 70:2437-2441, 1996 and the specific examples, Examples 5-7, below.

Since the protein-capture agents of at least some of the different patches on the array are different from each other, different solutions, each containing a different, preferably, affinity-tagged protein-capture agent, must be delivered to their individual patches. Solutions of protein-capture agents may be transferred to the appropriate patches via arrayers which are well-known in the art and even commercially available. For instance, microcapillary-based dispensing systems may be used. These dispensing systems are preferably automated and computer-aided. A description of and building instructions for an example of a microarrayer comprising an automated capillary system can be found on the internet at

<http://cmgm.stanford.edu/pbrown/array.html> and <http://cmgm.stanford.edu/pbrown/mguide/index.html>. The use of other microprinting techniques for transferring solutions containing the protein-capture agents to the agent-reactive patches is also possible. Ink-jet printer heads may also optionally be used for precise delivery of the protein-capture agents to the agent-reactive patches. Representative, non-limiting disclosures of techniques useful for depositing the protein-capture agents on the patches may be found, for example, in U.S. Patent Nos. 5,731,152 (stamping apparatus), 5,807,522 (capillary dispensing device), 5,837,860 (ink-jet printing technique, Hamilton 2200 robotic pipetting delivery system), and 5,843,767 (ink-jet printing technique, Hamilton 2200 robotic pipetting delivery system), all incorporated by reference herein.

(e) Adaptors.

Another embodiment of the array of the present invention comprises an adaptor that links the affinity tag to the protein-capture agent on the patches of the array. The additional spacing of the protein-capture agent from the surface of the substrate (or coating) that is afforded by the use of an adaptor is particularly advantageous if the protein-capture agent is a protein, since proteins are known to be prone to surface inactivation. The adaptor may optionally afford some additional advantages as well. For instance, the adaptor may help facilitate the attachment of the protein-capture agent to the affinity tag. In another embodiment, the adaptor may help facilitate the use of a particular detection technique with the array. One of ordinary skill in the art will be able to choose an adaptor which is appropriate for a given affinity tag. For instance, if the affinity tag is streptavidin, then the adaptor could be biotin that is chemically conjugated to the protein-capture agent which is to be immobilized.

In one embodiment, the adaptor comprises a protein. In another embodiment, the affinity tag, adaptor, and protein-capture agent together compose a fusion protein. Such a fusion protein may be readily expressed using standard recombinant DNA technology. Adaptors which are proteins are especially useful to increase the

solubility of the protein-capture agent of interest and to increase the distance between the surface of the substrate or coating and the protein-capture agent. Use of a protein adaptor can also be very useful in facilitating the preparative steps of protein purification by affinity binding prior to immobilization on the array. Examples of possible adaptor proteins include glutathione-S-transferase (GST), maltose-binding protein, chitin-binding protein, thioredoxin, green-fluorescent protein (GFP). GFP can also be used for quantification of surface binding. In a preferred embodiment, when the protein-capture agent is an antibody moiety comprising the Fc region, the adaptor is a polypeptide, such as protein G, protein A, or recombinant protein A/G (a gene fusion product secreted from a non-pathogenic form of *Bacillus* which contains four Fc binding domains from protein A and two from protein G).

Figure 7 shows a cross section of a patch on one particular embodiment of the invention array. The patch comprises a protein-capture agent 10 immobilized on a monolayer 7 via both an affinity tag 8 and an adaptor 9. The monolayer 7 rests on a coating 5. An interlayer 6 is used between the coating 5 and the substrate 3.

(f) Preparation of the protein-capture agents of the array.

The protein-capture agents used on the array may be produced by any of the variety of means known to those of ordinary skill in the art. In a preferred embodiment of the invention, the protein-capture agents are proteins, and in an especially preferred embodiment, the protein-capture agents are antibodies or antibody fragments. Therefore, methods of preparing these types of possible protein-capture agents are emphasized here.

In preparation for immobilization to the arrays of the present invention, the antibody moiety, or any other protein-capture agent which is a protein or polypeptide, can optionally be expressed from recombinant DNA either *in vivo* or *in vitro*. The cDNA of the antibody or antibody fragment or other protein-capture agent is cloned into an expression vector (many examples of which are commercially available) and introduced into cells of the appropriate organism for

expression. A broad range of host cells and expression systems may be used to produce the antibodies and antibody fragments, or other proteins, which serve as the protein-capture agents on the array. Expression *in vivo* may be done in bacteria (for example, *Escherichia coli*), plants (for example, *Nicotiana tabacum*), lower eukaryotes (for example, *Saccharomyces cerevisiae*, *Saccharomyces pombe*, *Pichia pastoris*), or higher eukaryotes (for example, baculovirus-infected insect cells, insect cells, mammalian cells). For *in vitro* expression PCR-amplified DNA sequences are directly used in coupled *in vitro* transcription/translation systems (for instance: *Escherichia coli* S30 lysates from T7 RNA polymerase expressing, preferably protease-deficient strains; wheat germ lysates; reticulocyte lysates (Promega, Pharmacia, Panvera)). The choice of organism for optimal expression depends on the extent of post-translational modifications (*i.e.*, glycosylation, lipid-modifications) desired. The choice of expression system also depends on other issues, such as whether an intact antibody is to be produced or just a fragment of an antibody (and which fragment), since disulfide bond formation will be affected by the choice of a host cell. One of ordinary skill in the art will be able to readily choose which host cell type is most suitable for the protein-capture agent and application desired.

DNA sequences encoding affinity tags and adaptors can be engineered into the expression vectors such that the protein-capture agent genes of interest can be cloned in frame either 5' or 3' of the DNA sequence encoding the affinity tag and adaptor protein.

The expressed protein-capture agents are purified by affinity chromatography using commercially available resins.

Preferably, production of a plurality of protein-capture agents involves parallel processing from cloning to protein expression and protein purification. cDNAs for the protein-capture agent of interest will be amplified by PCR using cDNA libraries or expressed sequence tags (EST) clones as templates. For *in vivo* expression of the proteins, cDNAs can be cloned into commercial expression vectors (Qiagen, Novagen, Clontech) and introduced into an appropriate organism

for expression (see above). For *in vitro* expression PCR-amplified DNA sequences are directly used in coupled *in vitro* transcription/translation systems (see above).

*Escherichia coli*-based protein expression is generally the method of choice for soluble proteins that do not require extensive post-translational modifications for activity. Extracellular or intracellular domains of membrane proteins will be fused to protein adaptors for expression and purification.

The entire approach can be performed using 96-well assay plates. PCR reactions are carried out under standard conditions. Oligonucleotide primers contain unique restriction sites for facile cloning into the expression vectors. Alternatively, the TA cloning system (Clontech) can be used. The expression vectors contain the sequences for affinity tags and the protein adaptors. PCR products are ligated into the expression vectors (under inducible promoters) and introduced into the appropriate competent *Escherichia coli* strain by calcium-dependent transformation (strains include: XL-1 blue, BL21, SG13009(lon-)). Transformed *Escherichia coli* cells are plated and individual colonies transferred into 96-array blocks. Cultures are grown to mid-log phase, induced for expression, and cells collected by centrifugation. Cells are resuspended containing lysozyme and the membranes broken by rapid freeze/thaw cycles, or by sonication. Cell debris is removed by centrifugation and the supernatants transferred to 96-tube arrays. The appropriate affinity matrix is added, the protein-capture agent of interest is bound and nonspecifically bound proteins are removed by repeated washing steps using 12 - 96 pin suction devices and centrifugation. Alternatively, magnetic affinity beads and filtration devices can be used (Qiagen). The proteins are eluted and transferred to a new 96-well array. Protein concentrations are determined and an aliquot of each protein-capture agent is spotted onto a nitrocellulose filter and verified by Western analysis using an antibody directed against the affinity tag on the protein-capture agent. The purity of each sample is assessed by SDS-PAGE and Silver staining or mass spectrometry. The protein-capture agents are then snap-frozen and stored at -80°C.

*Saccharomyces cerevisiae* allows for the production of glycosylated protein-capture agents such as antibodies or antibody fragments. For production in *Saccharomyces cerevisiae*, the approach described above for *Escherichia coli* can be used with slight modifications for transformation and cell lysis.

Transformation of *Saccharomyces cerevisiae* is by lithium-acetate and cell lysis is either by lyticase digestion of the cell walls followed by freeze-thaw, sonication or glass-bead extraction. Variations of post-translational modifications can be obtained by using different yeast strains (i.e., *Saccharomyces pombe*, *Pichia pastoris*).

One aspect of the baculovirus system is the array of post-translational modifications that can be obtained, although antibodies and other proteins produced in baculovirus contain carbohydrate structures very different from those produced by mammalian cells. The baculovirus-infected insect cell system requires cloning of viruses, obtaining high titer stocks and infection of liquid insect cell suspensions (cells such as SF9, SF21).

Mammalian cell-based expression requires transfection and cloning of cell lines. Either lymphoid or non-lymphoid cell may be used in the preparation of antibodies and antibody fragments. Soluble proteins such as antibodies are collected from the medium while intracellular or membrane bound proteins require cell lysis (either detergent solubilization, freeze-thaw). The protein-capture agents can then be purified analogous to the procedure described for *Escherichia coli*.

For *in vitro* translation the system of choice is *Escherichia coli* lysates obtained from protease-deficient and T7 RNA polymerase overexpressing strains. *Escherichia coli* lysates provide efficient protein expression (30-50 µg/ml lysate). The entire process is carried out in 96-well arrays. Antibody genes or other protein-capture agent genes of interest are amplified by PCR using oligonucleotides that contain the gene-specific sequences containing a T7 RNA polymerase promoter and binding site and a sequence encoding the affinity tag. Alternatively, an adaptor protein can be fused to the gene of interest by PCR.



Amplified DNAs can be directly transcribed and translated in the *Escherichia coli* lysates without prior cloning for fast analysis. The antibody fragments or other proteins are then isolated by binding to an affinity matrix and processed as described above.

Alternative *in vitro* translation systems which may be used include wheat germ extracts and reticulocyte extracts. *In vitro* synthesis of membrane proteins or post-translationally modified proteins will require reticulocyte lysates in combination with microsomes.

In one embodiment of the invention, the protein-capture agents on the array are monoclonal antibodies. The production of monoclonal antibodies against specific protein targets is routine using standard hybridoma technology. In fact, numerous monoclonal antibodies are available commercially. The preparation and use of an array of monoclonal antibodies is illustrated in the specific example, Example 8, below.

As an alternative to obtaining antibodies or antibody fragments by cell fusion or from continuous cell lines, the antibody moieties may be expressed in bacteriophage. Such antibody phage display technologies are well known to those skilled in the art. The bacteriophage expression systems allow for the random recombination of heavy- and light-chain sequences, thereby creating a library of antibody sequences which can be selected against the desired antigen. The expression system can be based on bacteriophage  $\lambda$  or, more preferably, on filamentous phage. The bacteriophage expression system can be used to express Fab fragments, Fv's with an engineered intermolecular disulfide bond to stabilize the V<sub>H</sub>-V<sub>L</sub> pair (dsFv's), scFvs, or diabody fragments.

The antibody genes of the phage display libraries may be from pre-immunized donors. For instance, the phage display library could be a display library prepared from the spleens of mice previously immunized with a mixture of proteins (such as a lysate of human T-cells). Immunization can optionally be used to bias the library to contain a greater number of recombinant antibodies reactive towards a specific set of proteins (such as proteins found in human T-cells). Alternatively,

the library antibodies may be derived from naive or synthetic libraries. The naive libraries have been constructed from spleens of mice which have not been contacted by external antigen. In a synthetic library, portions of the antibody sequence, typically those regions corresponding to the complementarity determining regions (CDR) loops, have been mutagenized or randomized. The phage display method involves batch-cloning the antibody gene library into a phage genome as a fusion to the gene encoding one of the phage coat proteins (pIII, pVI, or pVIII). The pIII phage protein gene is preferred. When the fusion product is expressed it is incorporated into the mature phage coat. As a result, the antibody is displayed as a fusion on the surface of the phage and is available for binding and hence, selection, on a target protein. Once a phage particle is selected as bearing an antibody-coat protein fusion with the desired affinity towards the target protein, the genetic material within the phage particle which corresponds to the displayed antibody can be amplified and sequenced or otherwise analyzed.

In a preferred embodiment, a phagemid is used as the expression vector in the phage display procedures. A phagemid is a small plasmid vector that carries gene III with appropriate cloning sites and a phage packaging signal and contains both host and phage origins of replication. The phagemid is unable to produce a complete phage as the gene III fusion is the only phage gene encoded on the phagemid. A viable phage can be produced by infecting cells containing the phagemid with a helper phage containing a defective replication origin. A hybrid phage emerges which contains all of the helper phage proteins as well as the gene III-rAb fusion. The emergent phage contains the phagemid DNA only.

In a preferred embodiment of the invention, the recombinant antibodies used in phage display methods of preparing protein-capture agents for the arrays of the invention are expressed as genetic fusions to the bacteriophage gene III protein on a phagemid vector. For instance, the antibody variable regions encoding a single-chain Fv fragment can be fused to the amino terminus of the gene III protein on a phagemid. Alternatively, the antibody fragment sequence could be fused to the amino terminus of a truncated pIII sequence lacking the first two N-terminal

domains. The phagemid DNA encoding the antibody-pIII fusion is preferably packaged into phage particles using a helper phage such as M13KO7 or VCS-M13, which supplies all structural phage proteins.

To display Fab fragments on phage, either the light or heavy (Fd) chain is fused via its C-terminus to pIII. The partner chain is expressed without any fusion to pIII so that both chains can associate to form an intact Fab fragment.

Any method of selection may be used which separates those phage particles which do bind the target protein from those which do not. The selection method must also allow for the recovery of the selected phages. Most typically, the phage particles are selected on an immobilized target protein. Some phage selection strategies known to those skilled in the art include the following: panning on an immobilized antigen; panning on an immobilized antigen using specific elution; using biotinylated antigen and then selecting on a streptavidin resin or streptavidin-coated magnetic beads; affinity purification; selection on Western blots (especially useful for unknown antigens or antigens difficult to purify); *in vivo* selection; and pathfinder selection. If the selected phage particles are amplified between selection rounds, multiple iterative rounds of selection may optionally be performed.

Elution techniques will vary depending upon the selection process chosen, but typical elution techniques include washing with one of the following solutions: HCl or glycine buffers; basic solutions such as triethylamine; chaotropic agents; solutions of increased ionic strength; or DTT when biotin is linked to the antigen by a disulfide bridge. Other typical methods of elution include enzymatically cleaving a protease site engineered between the antibody and gene III, or by competing for binding with excess antigen or excess antibodies to the antigen.

A method for producing an array of antibody fragments therefore comprises first selecting recombinant bacteriophage which express antibody fragments from a phage display library. The recombinant bacteriophage are selected by affinity binding to a protein which is an expression product, or fragment thereof, of a cell or population of cells in an organism. (Iterative rounds of selection are possible,

but optional.) Next, at least one purified sample of an antibody fragment from a bacteriophage which was selected in the first step is produced. This antibody production step typically entails infecting *E. coli* cells with the selected bacteriophage. In the absence of helper phage, the selected bacteriophage then replicate as expressive plasmids without producing phage progeny. Alternatively, the antibody fragment gene of the selected recombinant bacteriophage is isolated, amplified, and then expressed in a suitable expression system. In either case, following amplification, the expressed antibody fragment of the selected and amplified recombinant bacteriophage is isolated and purified. In a third step of the method, the earlier steps of phage display selection and purified antibody fragment production are repeated using affinity binding to different proteins which are expression products, or fragments thereof, of the same cell or population of cells as before until the desired plurality of purified samples of different antibodies with different binding pairs are produced. In a final step of the method, the antibody fragment of each different purified sample is immobilized onto an organic thinfilm on a separate patch on the surface of a substrate to form a plurality of patches of antibody fragments on discrete, known regions of the substrate surface covered by organic thinfilm.

For instance, to generate an antibody array with antibody fragments against known protein targets, open reading frames of the known protein targets identified in DNA databases are amplified by polymerase chain reaction and transcribed and translated *in vitro* to produce proteins on which a recombinant bacteriophage expressing single-chain antibody fragments are selected. Once selected, the antibody fragment sequence of the selected bacteriophage is amplified (typically using the polymerase chain method) and recloned into a desirable expression system. The expressed antibody fragments are purified and then printed onto organic thinfilms on substrates to form the high density arrays.

In another embodiment of the invention, a method for producing an array of protein-capture agents is provided which comprises first selecting protein-capture agents from a library of protein-capture agents, where the protein-capture agents

are selected by their affinity binding to the proteins from a cellular extract or body fluid. Preferably, the proteins are from a cellular extract. The proteins from the cellular extract or body fluid would typically be immobilized prior to the selection step. Suitable methods of immobilization such as crosslinking of the proteins to a resin are well known to one of ordinary skill in the art. The next step of this method comprises producing a plurality of purified samples of the selected protein-capture agents. The protein-capture agent of each different purified sample is immobilized onto an organic thinfilm on a separate patch on the surface of a substrate to form a plurality of patches of protein-capture agents on discrete, known regions of the substrate surface covered by organic thinfilm.

This method of array preparation optionally also comprises the additional step of biasing the library of protein-capture agents by eliminating from the library those protein-capture agents which bind certain proteins, such as the proteins of a second cellular extract, wherein the protein-capture agents which are eliminated are removed from the library by their binding affinity to those certain proteins. This step of biasing the library may optionally occur after the selection step by affinity binding to the protein, but more typically, it occurs prior to that selection step. The order of the selecting and biasing steps will depend on the nature of the selection and elution procedures used in the method. One of ordinary skill in the art will readily be able to determine an appropriate series of steps.

In one embodiment of the optional step of biasing the library of protein-capture agents, the library is biased to eliminate protein-capture agents that recognize common proteins or proteins of non-interest. This is typically achieved by passing the library over an affinity surface, such as a chromatography column, containing cross-linked proteins of non-interest. The "flowthrough" containing protein-capture agents that did not react with the affinity surface is collected. This procedure enriches the library for protein-capture agents which bind proteins of interest or proteins specific to the cell to be assayed. For instance, if the library is derived from a specific cell type such as a human T-cell, the library may optionally be biased by passing it over an affinity surface which contains proteins

prepared from a lysate of human fibroblasts or bacterial proteins to enrich the library for protein-capture agents which bind proteins specifically present in fibroblasts.

In a preferred embodiment of the method of preparing the array of protein-capture agents described above, the protein-capture agents are antibody fragments displayed on the surface of recombinant bacteriophages and the library of protein-capture agents is a phage display library. Therefore, a method for producing an antibody array comprises first selecting recombinant bacteriophage expressing antibody fragments from a phage display library, where the bacteriophage are selected by affinity binding to immobilized proteins of a body fluid, or more preferably, a cellular extract. The next step of this method comprises producing a plurality of purified samples of antibody fragments expressed by the selected recombinant bacteriophage. Preferably, antibody fragments which specifically bind more than 1000 of the proteins of the cellular extract are produced in this manner. In a final step of the method, the antibody fragment of each different purified sample is immobilized onto an organic thinfilm on a separate patch on the surface of a substrate to form a plurality of patches of antibody fragments on discrete known regions of the substrate surface. One specific example of this method is outlined in Example 6, below. Again, this method optionally also comprises the additional step of biasing the phage display library by eliminating from the library those bacteriophage displaying antibody fragments which bind certain proteins, such as the proteins of a second cellular extract. The bacteriophage which are eliminated are removed from the library by the binding affinity of their displayed antibody fragments to the certain proteins.

For instance, a method of preparing an antibody array optionally begins with a phage display library prepared from RNA isolated from the spleens of mice previously immunized with a lysate of human T-cells. The phage library is then passed over a column or affinity surface comprising proteins from the lysates of background cells such as human fibroblasts which have been cross-linked to a surface or resin. The phage remaining in the flowthrough solution from the first

column/affinity surface is then passed over a second affinity surface, such as a chromatography column, containing cross-linked proteins prepared from a lysate of human T-cells. The flowthrough solution from the second column/affinity surface is then discarded since this solution contains phage which displays recombinant antibodies that did not react with the second affinity surface. Phage which specifically react with the second affinity surface and remain bound to the second affinity surface are then collected by elution. Elution can be achieved by lowered pH (2.0), increased ionic strength, or proteolytic release by a specific proteolytic cut site genetically engineered between the displayed recombinant antibody and the gene III protein of the phage. In a next step of the method, the eluted phage are separated into isolated plaques by plating and then propagated as separate cultures. Periplasmic fractions from the separate cultures are prepared and the corresponding recombinant antibodies purified. The purified recombinant antibodies are then dispensed into separate patches on a 2-D array where they are immobilized onto an organic thinfilm.

Methods of preparing an array of protein-capture agents where the protein-capture agents have been selected against the proteins of a cellular extract, or a body fluid, create arrays of protein-capture agents where all of the binding partners of the arrays are not initially known. The primary information provided by binding of proteins to these types of arrays is contained in the pattern of protein abundance. Once interesting patches on an array have been identified by comparison of the protein expression pattern to that of a control (for instance, it may be observed that there is a significant increase in the amount of protein bound to a patch of the array following exposure of a cell to a certain set of conditions), the identity of the protein ligand binding to a particular patch on the array can be assessed by affinity purification of the protein ligand followed by microsequencing and/or mass spectrometry or the like.

An alternative method for producing an array of protein-capture agents comprises: selecting protein-capture agents from a library of protein-capture agents, wherein the protein-capture agents are selected by their binding affinity to

proteins expressed by a cDNA expression library; producing a plurality of purified samples of the selected protein-capture agents; and immobilizing each different purified protein-capture agent onto an organic thinfilm on a separate patch on the surface of a substrate to form a plurality of patches on discrete, known regions of the substrate surface covered by organic thinfilm

This method also optionally comprises the additional step of biasing the protein-capture agent library by eliminating from the library those protein-capture agents which bind certain proteins, such as the proteins of a cellular extract, wherein the protein-capture agents which are eliminated are removed from the library by their binding affinity to said certain proteins. In most cases, the proteins which are used to subtract protein-capture agents from the library of protein-capture agents would be immobilized. This step of biasing the library may optionally occur after the selection step by affinity binding to the proteins expressed by the cDNA expression library, but more typically, it occurs prior to that selection step. The order of these step will depend on the nature of the selection and elution steps. One of ordinary skill in the art will readily be able to determine an appropriate series of steps. In the optional step of biasing the library of protein-capture agents, the library is optionally biased to eliminate protein-capture agents that recognize common proteins or proteins of non-interest (as described above for a previous embodiment). Preferably, the method further comprises the additional step of identifying which individual selected protein-capture agents bind which individual proteins expressed by the cDNA expression library.

In another preferred embodiment of the the method, the protein-capture agents are antibody fragments displayed on the surface of recombinant bacteriophages and the library of protein-capture agents is a phage display library.

For instance, one example of a method of preparing an array of antibodies optionally begins with a phage display library prepared from RNA isolated from the spleens of mice previously immunized with a lysate of human T-cells. The phage library is then passed over a column or affinity surface comprising proteins



from the lysates of background cells such as human fibroblasts which have been cross-linked to a surface or resin. The phage remaining in the flowthrough solution from the first column/affinity surface is then collected. A cDNA expression library derived from message RNA (mRNA) isolated from human T-cells is prepared in which the expressed proteins from the expression library are genetically fused with an expression tag (such as a six histidine tag). The library is expanded and the tagged proteins are collectively expressed and purified. The pool of purified, tagged proteins from the cDNA expression library is cross-linked to an affinity surface, such as a chromatography column. The phage display library which passed through the first affinity surface or column is passed over the affinity surface bearing the immobilized proteins of the cDNA expression library. The flowthrough solution containing phage displaying recombinant antibodies that did not react with the affinity surface is discarded. Phage which specifically react with the affinity surface are collected by elution achieved by lowering the pH (2.0). Cells from the cDNA expression library are plated and a filter lift of the colonies is made using nitrocellulose or charged nylon filters. Reactive sites on the filter are blocked with a standard blocking solution and the filters are probed with the selected bacteriophage eluted off of the second column. The phage are visualized by reaction with a monoclonal antibody recognizing the gene VIII coat protein of the bacteriophage, conjugated to alkaline phosphatase. Reactive sites on the filter are cut out and the phage eluted from the filter pieces and propagated separately. The eluted phage are separated into isolated plaques and then propagated as separate cultures. Periplasmic fractions from the separate cultures are prepared and the corresponding recombinant antibodies purified. The purified recombinant antibodies are then dispensed onto separate patches of organic thinfilm on a 2-D array. Samples are reacted with the array and protein ligands with interesting differential abundance patterns (when compared to a control) are identified. Colonies on the original plate corresponding to the phage-reactive sites on the filter are propagated and the plasmids containing the cDNA sequenced to identify the protein ligands reactive with the recombinant antibodies of the phage.

In the preparation of the arrays of the invention, phage display methods analogous to those used for antibody fragments may be used for protein-capture agents other than antibody fragments as long as the protein-capture agent is composed of protein and is of suitable size to be incorporated into the phagemid or alternative vector and expressed as a fusion with a bacteriophage coat protein. Phage display techniques using non-antibody libraries typically make use of some type of protein host scaffold structure which supports the variable regions. For instance,  $\beta$ -sheet proteins,  $\alpha$ -helical handle proteins, and other highly constrained protein structures have been used as host scaffolds.

Alternative display vectors may also be used to produce the protein-capture agents, such as antibody moieties, which are printed on the arrays of the invention. Polysomes, stable protein-ribosome-mRNA complexes, can be used to replace live bacteriophage as the display vehicle for recombinant antibody fragments or other proteins (Hanes and Pluckthun, *Proc. Natl. Acad. Sci USA*, 94:4937-4942, 1997). The polysomes are formed by preventing release of newly synthesized and correctly folded protein from the ribosome. Selection of the polysome library is based on binding of the antibody fragments or other proteins which are displayed on the polysomes to the target protein. mRNA which encodes the displayed protein or antibody having the desired affinity for the target is then isolated. Larger libraries may be used with polysome display than with phage display.

In still another alternative method of preparing the protein-capture agents of the arrays of the invention, an alternative display method of selection such as lambda display (Mikawa *et al.*, *J. Mol. Biol.*, 262:21-30, 1996), bacterial display (Georgiou *et al.*, *Nat. Biotechnol.*, 15:29-34, 1997) or eukaryotic cell display may instead be used.

Furthermore, selection methods other than display methods may also be used in the preparation of protein-capture agents for the arrays of the invention. As indicated above, the protein-capture agents may be obtained by any *in vitro* or *in vivo* selection procedure known to those skilled in the art. In one embodiment of

the invention, protein-capture agents other than antibodies and antibody fragments are batch selected on the protein in cellular extracts. Such procedures generate a diversity of protein-capture agents which are highly suitable for applications in proteomics.

In alternative embodiments of the invention, the protein-capture agents are partially or wholly prepared by synthetic means. If the protein-capture agent is a protein, then methods of peptide synthetic or protein ligation may optionally be used to construct a protein from amino acid or polypeptide building blocks.

Protein-capture agents which are polynucleotides are readily prepared synthetically.

(g) Uses of the arrays.

The present invention also provides methods of using the invention arrays. In general, for a variety of applications including proteomics and diagnostics, the methods of the invention involve the delivery of the sample containing the proteins to be analyzed to the arrays. After the proteins of the sample have been allowed to interact with and become immobilized on the patches of the array comprising protein-capture agents with the appropriate biological specificity, the presence and/or amount of protein bound at each patch is then determined.

Use of one of the protein-capture agent arrays of the invention may optionally involve placing the two-dimensional array in a flowchamber with approximately 1-10 microliters of fluid volume per 25 mm<sup>2</sup> overall surface area. The cover over the array in the flowchamber is preferably transparent or translucent. In one embodiment, the cover may comprise Pyrex or quartz glass. In other embodiments, the cover may be part of a detection system that monitors interaction between the protein-capture agents immobilized on the array and protein in a solution such as a cellular extract. The flowchambers should remain filled with appropriate aqueous solutions to preserve protein activity. Salt, temperature, and other conditions are preferably kept similar to those of normal physiological conditions. Proteins in a fluid solution may be flushed into the flow

chamber as desired and their interaction with the immobilized protein-capture agents determined. Sufficient time must be given to allow for binding between the protein-capture agent and its binding partner to occur. The amount of time required for this will vary depending upon the nature and tightness of the affinity of the protein-capture agent for its binding partner. No specialized microfluidic pumps, valves, or mixing techniques are required for fluid delivery to the array. Alternatively, protein-containing fluid can be delivered to each of the patches of the array individually. For instance, in one embodiment, the regions of the substrate surface may be microfabricated in such a way as to allow integration of the array with a number of fluid delivery channels oriented perpendicular to the array surface, each one of the delivery channels terminating at the site of an individual protein-capture agent-coated patch.

The sample which is delivered to the array will typically be a fluid. In a preferred embodiment of the invention, the sample is a cellular extract or a body fluid. The sample to be assayed may optionally comprise a complex mixture of proteins, including a multitude of proteins which are not binding partners of the protein-capture agents of the array. If the proteins to be analyzed in the sample are membrane proteins, then those proteins will typically need to be solubilized prior to administration of the sample to the array. If the proteins to be assayed in the sample are proteins secreted by a population of cells in an organism, a sample which is derived from a body fluid is preferred. If the proteins to be assayed in the sample are intracellular, a sample which is a cellular extract is preferred. In one embodiment of the invention, the array may comprise protein-capture agents which bind fragments of the expression products of a cell or population of cells in an organism. In such a case, the proteins in the sample to be assayed may have been prepared by performing a digest of the protein in a cellular extract or a body fluid. In an alternative application of the array, the proteins from only specific fractions of a cell are collected for analysis in the sample.

In general, delivery of solutions containing proteins to be bound by the protein-capture agents of the array may optionally be preceded, followed, or accompanied

by delivery of a blocking solution. A blocking solution contains protein or another moiety which will adhere to sites of non-specific binding on the array. For instance, solutions of bovine serum albumin or milk may be used as blocking solutions.

It is understood that some proteins a sample which are not the intended binding partner of the protein-capture agents of a patch (and may, in fact, be the intended binding partner of another patch) on the array may still bind to the patch to some degree. Preferably, this type of binding only occurs to a very minor degree.

Also, it is understood that even when the correct binding partners are present in the solution being assayed, the binding partners will bind to the patch comprising their protein-capture agent with less than 100% efficiency.

A wide range of detection methods is applicable to the methods of the invention. As desired, detection may be either quantitative or qualitative. The invention array can be interfaced with optical detection methods such as absorption in the visible or infrared range, chemoluminescence, and fluorescence (including lifetime, polarization, fluorescence correlation spectroscopy (FCS), and fluorescence-resonance energy transfer (FRET)). Furthermore, other modes of detection such as those based on optical waveguides PCT Publication (WO 96/26432 and U.S. Patent No. 5,677,196), surface plasmon resonance, surface charge sensors, and surface force sensors are compatible with many embodiments of the invention. Alternatively, technologies such as those based on Brewster Angle microscopy (BAM) (Schaaf *et al.*, *Langmuir*, 3:1131-1135 (1987)) and ellipsometry (U.S. Patent Nos. 5,141,311 and 5,116,121; Kim, *Macromolecules*, 22:2682-2685 (1984)) could be applied. Quartz crystal microbalances and desorption processes (see for example, U.S. Patent No. 5,719,060) provide still other alternative detection means suitable for at least some embodiments of the invention array. An example of an optical biosensor system compatible both with some arrays of the present invention and a variety of non-label detection principles including surface plasmon resonance, total internal reflection fluorescence (TIRF), Brewster Angle microscopy, optical waveguide lightmode

spectroscopy (OWLS), surface charge measurements, and ellipsometry can be found in U.S. Patent No. 5,313,264.

Although non-label detection methods are generally preferred, some of the types of detection methods commonly used for traditional immunoassays which require the use of labels may be applied to the arrays of the present invention. These techniques include noncompetitive immunoassays, competitive immunoassays, and dual label, ratiometric immunoassays. These particular techniques are primarily suitable for use with the arrays of protein-capture agents when the number of different protein-capture agents with different specificity is small (less than about 100). In the competitive method, binding-site occupancy is determined indirectly. In this method, the protein-capture agents of the array are exposed to a labeled developing agent, which is typically a labeled version of the analyte or an analyte analog. The developing agent competes for the binding sites on the protein-capture agent with the analyte. The fractional occupancy of the protein-capture agents on different patches can be determined by the binding of the developing agent to the protein-capture agents of the individual patches. In the noncompetitive method, binding site occupancy is determined directly. In this method, the patches of the array are exposed to a labeled developing agent capable of binding to either the bound analyte or the occupied binding sites on the protein-capture agent. For instance, the developing agent may be a labeled antibody directed against occupied sites (*i.e.*, a "sandwich assay"). Alternatively, a dual label, ratiometric, approach may be taken where the protein-capture agent is labeled with one label and the second, developing agent is labeled with a second label (Ekins, *et al.*, *Clinica Chimica Acta.*, 194:91-114, 1990). Many different labeling methods may be used in the aforementioned techniques, including radioisotopic, enzymatic, chemiluminescent, and fluorescent methods.

Fluorescent methods are preferred.

Figure 8 shows a schematic diagram of one type of fluorescence detection unit which may be used to monitor interaction of immobilized protein-capture agents of an array with a protein analyte. In the illustrated detection unit, the array of

protein-capture agents 21 is positioned on a base plate 20. Light from a 100W mercury arc lamp 25 is directed through an excitation filter 24 and onto a beam splitter 23. The light is then directed through a lens 22, such as a Micro Nikkor 55 mm 1:2.8 lens, and onto the array 21. Fluorescence emission from the array returns through the lens 22 and the beam splitter 23. After next passing through an emission filter 26, the emission is received by a cooled CCD camera 27, such as the Slowscan TE/CCD-1024SF&SB (Princeton Instruments). The camera is operably connected to a CPU 28 which is in turn operably connected to a VCR 29 and a monitor 30.

Figure 9 shows a schematic diagram of an alternative detection method based on ellipsometry. Ellipsometry allows for information about the sample to be determined from the observed change in the polarization state of a reflected light wave. Interaction of a protein analyte with a layer of immobilized protein-capture agents on a patch results in a thickness change and alters the polarization status of a plane-polarized light beam reflected off the surface. This process can be monitored *in situ* from aqueous phase and, if desired, in imaging mode. In a typical setup, monochromatic light (e.g. from a He-Ne laser, 30) is plane polarized (polarizer 31) and directed onto the surface of the sample and detected by a detector 35. A compensator 32 changes the elliptically polarized reflected beam to plane-polarized. The corresponding angle is determined by an analyzer 33 and then translated into the ellipsometric parameters Psi and Delta which change upon binding of protein with the protein-capture agents. Additional information can be found in Azzam, et al., *Ellipsometry and Polarized Light*, North-Holland Publishing Company: Amsterdam, 1977.

The arrays of the present invention are particularly useful for proteomics. Those arrays which comprise significant numbers of protein-capture agents of different specificity on separate patches can bind significant numbers of proteins which are expression products, or fragments thereof, of a cell or population of cells in an organism and are particularly suitable for use in applications involving proteomics. For instance, an array with at least about  $10^3$  and up to about  $10^5$

different protein-capture agents such as antibodies or antibody fragments can provide a highly comprehensive picture of the protein content of the cell under a specific set of conditions.

In one embodiment of the invention, a method of assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism, is provided which comprises the following steps: first, delivering the sample to an array of spatially distinct patches of different protein-capture agents under conditions suitable for protein binding, wherein each of the proteins being assayed is a binding partner of the protein-capture agent of at least one patch on the array; next, optionally washing said array to remove unbound or nonspecifically bound components of the sample from the array; and in a final step, detecting, either directly or indirectly, for the presence or amount of protein bound to each patch of the array.

In another embodiment of the invention, a method of assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism, comprises first delivering the sample to the invention array of protein-capture agents under conditions suitable for protein binding, wherein each of the proteins being assayed is a binding partner of the protein-capture agent of at least one patch on the array. The first step may be followed by an optional step of washing the array with fluid to remove unbound or nonspecifically bound components of the sample from the array. Lastly, the presence or amount of protein bound to each patch is detected, either directly or indirectly.

A variety of different embodiments of the invention array of protein-capture agents may be used in the methods for assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism. Generally, preferred embodiments of these methods comprise the use of preferred arrays of the invention. For instance, in preferred embodiments of the method, the protein-



capture agents are antibodies or antibody fragments. In further preferred embodiments for assaying the different amounts of a plurality of proteins in a cell in parallel or the protein expression pattern of a cell, the plurality of patches on the array can bind at least about 100 or at least about  $10^3$  different proteins which are the expression products, or fragments thereof, of a cell or population of cells in an organism. Alternatively, the plurality of patches on the array used in the methods can bind at least about  $10^4$  different proteins which are the expression products, or fragments thereof, of a cell or population of cells in an organism. The methods of assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism, optionally comprise the additional step of further characterizing the protein bound to at least one patch of the array. This step is typically designed to identify the nature of the protein bound to the protein-capture agent of a particular patch. In some cases, the entire identity of the bound protein may not be known and the purpose of the further characterization may be the initial identification of the mass, sequence, structure and/or activity of the bound protein. In other cases, the basic identity of the protein may be known, but the post-translational modification, activation state, or some other feature of the protein may not be known. In one embodiment, the step of further characterizing the proteins involves measuring the activity of the proteins. Although in some cases it may be preferable to remove the protein from the patch before the step of further characterizing the protein is carried out, in other cases the protein can be further characterized while still bound to the patch. In still further embodiments, the protein-capture agents of the patch which binds a protein can be used to isolate and/or purify the protein from cells. The purified sample can then be characterized through traditional means such as microsequencing, mass spectrometry, and the like.

In another embodiment, the present invention provides a method of determining the protein expression pattern of a cell or population of cells in an organism. This method involves first delivering a sample containing expression products, or

fragments thereof, of the cell or population of cells to the protein-capture agent array of the invention under conditions suitable for protein binding. The presence and/or amount of protein bound to each patch can then be determined by a suitable detection means. The detection may be either direct or indirect.

Quantitative detection is typically preferred for this application (and for other proteomics applications). The method preferably further comprises an additional step before the detection step comprising washing the array to remove unbound or nonspecifically bound components of the sample from the array. The amount of protein bound to a patch of the array may optionally be determined relative to the amount of a second protein bound to a second patch of the array. The method of determining the protein expression pattern of a cell or a population of cells in an organism, optionally comprises the additional step of further characterizing the proteins bound to at least one patch of the array, as previously described above. In the method of assaying the protein expression pattern of a cell or population of cells in an organism, many of the targets of the protein-capture agents of the array may optionally be of unknown sequence, identity, and/or function. For instance, the antibodies of the array may have been prepared by selecting a phage display library by affinity binding to the immobilized proteins of a cellular extract which contains many unidentified proteins. If the protein bound by a protein-capture agent on a particular patch of an array is unknown, but is of interest, then that protein may optionally be later identified or characterized by first using the same protein-capture agent that was used on the array to isolate the protein in question from cells. The isolated binding partner from the cell can then be assayed directly for function and/or sequenced.

The arrays of protein-capture agents may also be used to compare the protein expression patterns of two cells or populations of cells. In this method, a sample containing expression products, or fragments thereof, of a first cell or population of cells is delivered to the invention array of protein-capture agents under conditions suitable for protein binding. In an analogous manner, a sample containing expression products, or fragments thereof, of a second cell or population

of cells to a second array, is delivered to a second array which is identical to the first array. Preferably, both arrays are then washed to remove unbound or nonspecifically bound components of the sample from the arrays. In a final step, the amounts of protein remaining bound to the patches of the first array are compared to the amounts of protein remaining bound to the corresponding patches of the second array. If it is desired to determine the differential protein expression pattern of two cells or populations of cells, for instance, then the amount of protein bound to the patches of the first array may be subtracted from the amount of protein bound to the corresponding patches of the second array.

Methods of comparing the protein expression of two cells or populations of cells are particularly useful for the understanding of biological processes. For instance, using these methods, the protein expression patterns of identical cells or closely related cells exposed to different conditions can be compared. Most typically, the protein content of one cell or population of cells is compared to the protein content of a control cell or population of cells. For instance, in one embodiment of the invention, one of the cells or populations of cells is neoplastic and the other cell is not. In another embodiment, one of the two cells or populations of cells being assayed is infected with a pathogen. Alternatively, one of the two cells or populations of cells has been exposed to a stressor and the other cell or population of cells serves as a control. The stressor may optionally be chemical, environmental, or thermal. One of the two cells may optionally be exposed to a drug or a potential drug and its protein expression pattern compared to a control cell.

Such methods of assaying differential gene expression at the protein level are useful in the identification and validation of new potential drug targets as well as for drug screening. For instance, the method may be used to identify a protein which is overexpressed in tumor cells, but not in normal cells. This protein may be a target for drug intervention. Inhibitors to the action of the overexpressed protein can then be developed. Alternatively, antisense strategies to inhibit the overexpression may be developed. In another instance, the protein expression

pattern of a cell, or population of cells, which has been exposed to a drug or potential drug can be compared to that of a cell, or population of cells, which has not been exposed to the drug. This comparison will provide insight as to whether or not the drug has had the desired effect on a target protein (drug efficacy) and whether other proteins of the cell, or population of cells, have also been affected (drug specificity).

The arrays of the present invention are also suitable for diagnostic applications and suitable for use in diagnostic devices. The high density of the antibodies on some arrays of the present invention enables a large number of different, antibody-based diagnostic tests to be formatted onto a single biochip. The protein-capture agents on the invention array can be used to evaluate the status of a disease condition in a tissue, such as a tumor, where the expression levels of certain proteins in the cells of the tissue is known to be indicative of a particular type of disease condition or stage of a disease condition. If certain patterns of protein expression are not previously known to be indicative of a disease state, the protein-capture agent arrays of the invention can then first be used to establish this information.

Accordingly, in one embodiment, the invention provides a method of evaluating a disease condition in a tissue of an organism comprising first contacting the invention array of protein-capture agents with a sample comprising the expression products, or fragments thereof, of the cells of the tissue being evaluated, wherein the contacting occurs under conditions suitable for protein binding and wherein the binding partners of a plurality of protein-capture agents on the array include proteins which are expression products, or fragment thereof, of the cells of the tissue and whose expression levels are indicative of the disease condition. The method next comprises detecting, either directly or indirectly, for the presence of protein to each patch. In a preferred embodiment, the method further comprises the step of washing the array to remove unbound or nonspecifically bound components of the sample from the array. In such a method, the array will typically comprise protein-capture agents which bind those proteins whose

presence, absence, or relative amount in cells is known to be indicative of a particular type of disease condition or state of a disease condition. For instance, the plurality of proteins being assayed in such a method may include such proteins as HER2 protein or prostate-specific antigen (PSA).

(h) Examples.

The following specific examples are intended to illustrate the invention and should not be construed as limiting the scope of the claims:

Example 1. Fabrication of a two-dimensional array by photolithography.

In a preferred embodiment of the invention, two-dimensional arrays are fabricated onto the substrate material via standard photolithography and/or thin film deposition. Alternative techniques include microcontact printing. Usually, a computer-aided design pattern is transferred to a photomask using standard techniques, which is then used to transfer the pattern onto a silicon wafer coated with photoresist.

In a typical example, the array ("chip") with lateral dimensions of 10 x 10 mm comprises squared patches of a bioreactive layer (here: gold as the coating on a silicon substrate) each 0.1 x 0.1 mm in size and separated by hydrophobic surface areas with a 0.2 mm spacing. 4" diameter Si(100) wafers (Virginia Semiconductor) are used as bulk materials. Si(100) wafers are first cleaned in a 3:1 mixture of H<sub>2</sub>SO<sub>4</sub>, conc.: 30% H<sub>2</sub>O<sub>2</sub> (90°C, 10 min), rinsed with deionized water (18 MΩcm), finally passivated in 1% aqueous HF, and singed at 150°C for 30 min to become hydrophobic. The wafer is then spincoated with photoresist (Shipley 1813), prebaked for 25 minutes at 90°C, exposed using a Karl Suss contact printer and developed according to standard protocols. The wafer is then dried and postbaked at 110°C for 25 min. In the next step, the wafer is primed with a titanium layer of 20 nm thickness, followed by a 200 nm thick gold layer. Both layers were deposited using electron-beam evaporation (5 Å/s). After resist stripping and a short plasma treatment, the gold patches can be further chemically

modified to achieve the desired bioreactive and biocompatible properties (see Example 3, below).

Example 2. Fabrication of a two-dimensional array by deposition through a hole mask.

In another preferred embodiment the array of gold patches is fabricated by thin film deposition through a hole mask which is in direct contact with the substrate. In a typical example, Si(100) wafers are first cleaned in a 3:1 mixture of H<sub>2</sub>SO<sub>4</sub>, conc.: 30% H<sub>2</sub>O<sub>2</sub> (90°C, 10 min), rinsed with deionized water (18 MΩcm), finally passivated in 1% aqueous HF and singed at 150°C for 30 min to become hydrophobic. The wafer is then brought into contact with a hole mask exhibiting the positive pattern of the desired patch array. In the next step, the wafer is primed with a titanium layer of 20 nm thickness, followed by a 200 nm thick gold layer. Both layers were deposited using electron-beam evaporation (5 Å/s). After removal of the mask, the gold patches can be further chemically modified to achieve the desired bioreactive and biocompatible properties (see Example 3, below).

Example 3. Synthesis of an aminoreactive monolayer molecule (following the procedure outlined in Wagner *et al.*, *Biophys. J.*, 1996, 70:2052-2066).

*General.* <sup>1</sup>H- and <sup>13</sup>C-NMR spectra are recorded on Bruker instruments (100 to 400 MHz). Chemical shifts (δ) are reported in ppm relative to internal standard ((CH<sub>3</sub>)<sub>4</sub>Si, δ = 0.00 (<sup>1</sup>H- and <sup>13</sup>C-NMR)). FAB-mass spectra are recorded on a VG-SABSEQ instrument (Cs<sup>+</sup>, 20 keV). Transmission infrared spectra are obtained as dispersions in KBr on an FTIR Perkin-Elmer 1600 Series instrument. Thin-layer chromatography (TLC) is performed on precoated silica gel 60 F254 plates (MERCK, Darmstadt, FRG), and detection was done using Cl<sub>2</sub>/toluidine, PdCl<sub>2</sub> and UV-detection under NH<sub>3</sub>-vapor. Medium pressure liquid chromatography (MPLC) is performed on a Labomatic MD-80 (LABOMATIC INSTR. AG, Allschwil, Switzerland) using a Buechi column (460x36 mm;

BUECHI, Flawil, Switzerland), filled with silica gel 60 (particle size 15-40  $\mu\text{m}$ ) from Merck.

*Synthesis of 11,11'-dithiobis(succinimidylundecanoate)* (DSU). Sodium thiosulfate (55.3 g, 350 mmol) is added to a suspension of 11-bromo-undecanoic acid (92.8 g, 350 mmol) in 50% aqueous 1,4-dioxane (1000 ml). The mixture is heated at reflux (90°C) for 2 h until the reaction to the intermediate Bunte salt was complete (clear solution). The oxidation to the corresponding disulfide is carried out *in situ* by adding iodine in portions until the solution retained with a yellow to brown colour. The surplus of iodine is retitrated with 15% sodium pyrosulfite in water. After removal of 1,4-dioxane by rotary evaporation the creamy suspension is filtered to yield product *11,11'-dithiobis(undecanoic acid)*. Recrystallization from ethyl acetate/THF provides a white solid (73.4 g, 96.5%): mp 94°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$  /  $\text{CD}_3\text{OD}$  95 : 5):  $\delta$  2.69 (t, 2H,  $J = 7.3$  Hz), 2.29 (t, 2H,  $J = 7.5$  Hz), 1.76-1.57 (m, 4H), and 1.40-1.29 (m, 12H); FAB-MS ( $\text{Cs}^+$ , 20 keV):  $m/z$  (relative intensity) 434 (100,  $\text{M}^+$ ). Anal. Calcd. for  $\text{C}_{22}\text{H}_{42}\text{O}_4\text{S}_2$ : C, 60.79; H, 9.74; S, 14.75. Found: C, 60.95; H, 9.82; S, 14.74. To a solution of *11,11'-dithiobis(undecanoic acid)* (1.0 g, 2.3 mmol) in THF (50 ml) is added N-hydroxysuccinimide (0.575 g, 5 mmol) followed by DCC (1.03 g, 5 mmol) at 0°C. After the reaction mixture is allowed to warm to 23°C and is stirred for 36 h at room temperature, the dicyclohexylurea (DCU) is filtered. Removal of the solvent under reduced pressure and recrystallization from acetone/hexane provides *11,11'-dithiobis(succinimidylundecanoate)* as a white solid. Final purification is achieved by medium pressure liquid chromatography (9 bar) using silica gel and a 2:1 mixture of ethyl acetate and hexane. The organic phase is concentrated and dried in vacuum to afford *11,11'-dithiobis(succinimidylundecanoate)* (1.12 g, 78%): mp 95°C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.83 (s, 4H), 2.68 (t, 2H,  $J = 7.3$  Hz), 2.60 (t, 2H,  $J = 7.5$  Hz), 1.78-1.63 (m, 4H), and 1.43-1.29 (m, 12H); FAB-MS ( $\text{Cs}^+$ , 20 keV):  $m/z$  (relative intensity) 514 (100), 628 (86,  $\text{M}^+$ ). Anal. Calcd. for  $\text{C}_{30}\text{H}_{48}\text{N}_2\text{O}_8\text{S}_2$ : C, 57.30; H, 7.69; N, 4.45; S, 10.20. Found: C, 57.32; H, 7.60; N, 4.39; S, 10.25.

Example 4. Formation of an aminoreactive monolayer on gold (following the procedure of Wagner *et al.*, *Biophys. J.*, 1996, 70:2052-2066).

Monolayers based on 11,11'-dithiobis(succinimidylundecanoate) (DSU) can be deposited on Au(111) surfaces of substrates described under Examples 1 and 2 by immersing them into a 1 mM solution of DSU in chloroform at room temperature for 1 hour. After rinsing with 10 volumes of solvent, the N-hydroxysuccinimidyl-terminated monolayer is dried under a stream of nitrogen and immediately used for immobilization of the protein-capture agents.

Example 5. Formation and use of an array of immobilized Fab' antibody fragments to detect concentrations of soluble proteins prepared from cultured mammalian cells.

Collections of IgG antibodies are purchased from commercial sources (*e.g.* Pierce, Rockford, IL). The antibodies are first purified by affinity chromatography based on binding to immobilized protein A. The antibodies are diluted 1:1 in binding buffer (0.1 M Tris-HCl, 0.15 M NaCl, pH 7.5). A 2 ml minicolumn containing a gel with immobilized protein A is prepared. (Hermanson, *et al.*, *Immobilized Affinity Ligand Techniques*, Academic Press, San Diego, 1992.) The column is equilibrated with 10 ml of binding buffer. Less than 10 mg of immunoglobulin is applied to each 2 ml minicolumn and the column is washed with binding buffer until the absorbance at 280 nm is less than 0.02. The bound immunoglobulins are eluted with 0.1 M glycine, 0.15 M NaCl, pH 2.8, and immediately neutralized with 1.0 M Tris-HCl, pH 8.0 to 50 mM final concentration and then dialyzed against 10 mM sodium phosphate, 0.15 M NaCl, pH 7.2 and stored at 4°C.

The purified immunoglobulin are digested with immobilized pepsin. Pepsin is an acidic endopeptidase and hydrolyzes proteins favorably adjacent to aromatic and dicarboxylic L-amino acid residues. Digestion of IgG with pepsin generates intact F(ab')<sub>2</sub> fragments. Immobilized pepsin gel is washed with digestion buffer; 20 mM sodium acetate, pH 4.5. A solution of purified IgG at 10 mg/ml is added to the immobilized pepsin gel and incubated at 37°C for 2 hours. The reaction is



neutralized by the addition of 10 mM Tris-HCl, pH 7.5 and centrifuged to pellet the gel. The supernatant liquid is collected and applied to an immobilized protein A column, as described above, to separate the  $F(ab')_2$  fragments from the Fc and undigested IgG. The pooled  $F(ab')_2$  is dialyzed against 10 mM sodium phosphate, 0.15 M NaCl, pH 7.2 and stored at 4°C. The quantity of pooled, eluted  $F(ab')_2$  is measured by peak area absorbance at 280 nm.

The purified  $F(ab')_2$  fragments at a concentration of 10 mg/ml are reduced at 37°C for 1 hour in a buffer of 10 mM sodium phosphate, 0.15 M NaCl, 10 mM 2-mercaptoethylamine, 5 mM EDTA, pH 6.0. The Fab' fragments are separated from unsplit  $F(ab')_2$  fragments and concentrated by application to a Sephadex G-25 column ( $M_r = 46,000 - 58,000$ ). The pooled Fab' fragments are dialyzed against 10 mM sodium phosphate, 0.15 M NaCl, pH 7.2. The reduced Fab' fragments are diluted to 100 µg/ml and applied onto the bioreactive patches containing exposed aminoreactive functional groups using a computer-aided, capillary-based microdispensing system (for antibody immobilization procedures, see Dammer *et al.*, *Biophys. J.*, 70:2437-2441, 1996). After an immobilization period of 30 minutes at 30°C, the array is rinsed extensively with 10 mM sodium phosphate, 0.15 M NaCl, 5 mM EDTA, pH 7.0.

Transformed human cells grown in culture are collected by low speed centrifugation, briefly washed with ice-cold phosphate-buffered solution (PBS), and then resuspended in ice-cold hypotonic buffer containing DNase/RNase (10 µg/ml each, final concentration) and a mixture of protease inhibitors. Cells are transferred to a microcentrifuge tube, allowed to swell for 5 minutes, and lysed by rapid freezing in liquid nitrogen and thawing in ice-cold water. Cell debris and precipitates are removed by high-speed centrifugation and the supernatant is cleared by passage through a 0.45 µm filter. The cleared lysate is applied to the Fab' fragment array described above and allowed to incubate for 2 hours at 30°C. After binding the array is washed extensively with 10 mM sodium phosphate, 0.15 M NaCl, 5 mM EDTA, pH 7.0. The location and amount of bound proteins are determined by optical detection.

Example 6. Formation and use of an array of immobilized antibody fragments to detect concentrations of soluble proteins prepared from cultured mammalian cells. A combinatorial library of filamentous phage expressing scFv antibody fragments is generated based on the technique of McCafferty and coworkers; McCafferty, *et al.*, *Nature*, 1990, 348:552-554; Winter and Milstein, *Nature*, 1991, 349:293-299. Briefly, mRNA is purified from mouse spleens and used to construct a cDNA library. PCR fragments encoding sequences of the variable heavy and light chain immunoglobulin genes of the mouse are amplified from the prepared cDNA. The amplified PCR products are joined by a linker region of DNA encoding the 15 amino acid peptide (Gly<sub>4</sub>SerGly<sub>2</sub>CysGlySerGly<sub>4</sub>Ser) (SEQ ID NO: 1) and the resulting full-length PCR fragment is cloned into an expression plasmid (pCANTAB 5 E) in which the purification peptide tag (E Tag) has been replaced by a His<sub>6</sub> peptide (SEQ ID NO: 2). Electrocompetent TG1 *E. coli* cells are transformed with the expression plasmid by electroporation. The pCANTAB-transformed cells are induced to produced functional filamentous phage expressing scFv fragments by superinfection with M13KO7 helper phage. Cells are grown on glucose-deficient medium containing the antibiotics ampicillin (to select for cells with the phagemid) and kanamycin (to select for cells infected with M13KO7). In the absence of glucose, the lac promoter present on the phagemid is no longer repressed, and synthesis of the scFv-gene 3 fusion begins. Proteins from a cell lysate are adsorbed to the wells of a 96-well plate. Transformed human cells grown in culture are collected by low speed centrifugation and the cells are briefly washed with ice-cold PBS. The washed cells are then resuspended in ice-cold hypotonic buffer containing DNase/RNase (10 µg/ml each, final concentration) and a mixture of protease inhibitors, allowed to swell for 5 minutes, and lysed by rapid freezing in liquid nitrogen and thawing in ice-cold water. Cell debris and precipitates are removed by high-speed centrifugation and the supernatant is cleared by passage through a 0.45 µm filter. The cleared lysate is diluted to 10 µg/ml in dilution buffer; 20 mM PIPES, 0.15 M NaCl, 0.1 % CHAPS, 10%, 5 mM EDTA, 5 mM 2-mercaptoethanol, 2 mM DTT.

pH 7.2 and applied to the 96-plate wells. After immobilization for 1 hour at 30°C, the well is washed with the dilution buffer and then incubated with dilution buffer containing 10% nonfat dry milk to block unreacted sites. After the blocking step, the well is washed extensively with the dilution buffer.

Phage expressing displayed antibodies are separated from *E. coli* cells by centrifugation and then precipitated from the supernatant by the addition of 15% w/v PEG 8000, 2.5 M NaCl followed by centrifugation. The purified phage are resuspended in the dilution buffer containing 3% nonfat dry milk and applied to the well containing the immobilized proteins described above, and allowed to bind for 2 hours at 37°C, followed by extensive washing with the binding buffer. Phage are eluted from the well with an elution buffer; 20 mM PIPES, 1 M NaCl, 0.1 % CHAPS, 10%, 5 mM EDTA, 5 mM 2-mercaptoethanol, 2 mM DTT, pH 7.2. The well is then extensively washed with purge buffer; 20 mM PIPES, 2.5 M NaCl, 0.1 % CHAPS, 10%, 5 mM EDTA, 5 mM 2-mercaptoethanol, 2 mM DTT, pH 7.2. The well is then extensively washed with dilution buffer; 20 mM PIPES, 0.15 M NaCl, 0.1 % CHAPS, 10%, 5 mM EDTA, 5 mM 2-mercaptoethanol, 2 mM DTT, pH 7.2. The eluted phage solution is then re-applied to a new well containing adsorbed antigen and the panning enrichment is repeated 4 times. Finally, the phage are eluted from the well with 2M of NaCl in 20 mM PIPES, 0.1 % CHAPS, 10%, 5 mM EDTA, 5 mM 2-mercaptoethanol, 2 mM DTT, pH 7.2. Eluates are collected and mixed with log-phase TG1 cells, and grown at 37°C for 1 hour and then plated onto SOB medium containing ampicillin and glucose and allowed to grow for 12 – 24 hours.

Individual colonies are picked and arrayed into 96-well 2ml blocks containing SOB medium and M13KO7 helper phage and grown for 8 hours with shaking at 37°C. The phage are separated from cells by centrifugation and precipitated with PEG/NaCl as described above. Concentrated phage are used to infect HB2151 *E. coli*. *E. coli* TG1 produces a suppressor tRNA which allows readthrough (suppression) of an amber stop codon located between the scFv and phage gene 3 sequences of the pCANTAB 5 E plasmid. Infected HB2151 cells are selected on

medium containing ampicillin, glucose, and nalidixic acid. Cells are grown to mid-log and then centrifuged and resuspended in medium lacking glucose and growth continued. Soluble scFv fragments will accumulate in the cell periplasm. A periplasmic extract is prepared from pelleted cells by mild osmotic shock. The soluble scFv released into the supernatant is purified by affinity binding to Ni-NTA activated agarose and eluted with 10 mM EDTA.

The purified scFv antibody fragments are diluted to 100 µg/ml and applied onto the bioreactive patches with exposed aminoreactive groups using a computer-aided, capillary-based microdispensing system. After an immobilization period of 30 minutes at 30°C, the array is rinsed extensively with 10 mM sodium phosphate, 0.15 M NaCl, 5 mM EDTA, pH 7.0.

Transformed human cells grown in culture are collected by low speed centrifugation, briefly washed with ice-cold PBS, and then resuspended in ice-cold hypotonic buffer containing DNase/RNase (10 µg/ml each, final concentration) and mixture of protease inhibitors. Cells are transferred to a microcentrifuge tube, allowed to swell for 5 minutes, and lysed by rapid freezing in liquid nitrogen and thawing in ice-cold water. Cell debris and precipitates are removed by high-speed centrifugation and the supernatant is cleared by passage through a 0.45 µm filter. The cleared lysate is applied to the scFv fragment array described above and allowed to incubate for 2 hours at 30°C. After binding, the array is washed extensively with 0.1 M sodium phosphate, 0.15 M NaCl, 5 mM EDTA pH 7.0. The location and amount of bound proteins are determined by optical detection.

Patterns of binding are established empirically by testing dilutions of a control cell extract. Extracts from experimental cells are diluted to a series of concentrations and then tested against the array. Patterns of protein expression in the experimental cell lysates are compared to protein expression patterns in the control samples to identify proteins with unique expression profiles.

Example 7. Formation and use of an array of immobilized monoclonal antibodies to detect concentrations of soluble proteins prepared from cultured mammalian cells.

Collections of monoclonal antibodies are purchased from commercial suppliers as either raw ascities fluid or purified by chromatography over protein A, protein G, or protein L. If from raw ascites fluid, the antibodies are purified using a HiTrap Protein G or HiTrap Protein A column (Pharmacia) as appropriate for the immunoglobulin subclass and species. Prior to chromatography the ascites are diluted with an equal volume of 10 mM sodium phosphate, 0.9 % NaCl, pH 7.4 (PBS) and clarified by passage through a 0.22  $\mu$ m filter. The filtrate is loaded onto the column in PBS and the column is washed with two column volumes of PBS. The antibody is eluted with 100 mM Glycine-HCl, pH 2.7 (for protein G) or 100 mM citric acid, pH 3.0 (for protein A). The eluate is collected into 1/10 volume 1 M Tris-HCl, pH 8.0. The final pH is 7.5. Fractions containing the antibodies are confirmed by SDS-PAGE and then pooled and dialyzed against PBS.

The different samples of purified antibodies are each diluted to 100  $\mu$ g/ml. Each different antibody sample is applied to a separate patch of an array of aminoreactive monolayer patches (see Example 4, above) using a computer-aided, capillary-based microdispensing system. After an immobilization period of 30 minutes at 30°C, the array is rinsed extensively with 10 mM sodium phosphate, 0.15 M NaCl, 5 mM EDTA, pH 7.0.

Transformed human cells grown in culture are collected by low speed centrifugation, briefly washed with ice-cold PBS, and resuspended in ice-cold hypotonic buffer containing Dnase/Rnase (10  $\mu$ g/ml each, final concentration) and

a mixture of protease inhibitors. Cells are transferred to a microcentrifuge tube, allowed to swell for 5 minutes, and lysed by rapid freezing in liquid nitrogen and thawing in ice-cold water. Cell debris and precipitates are removed by high-speed centrifugation and the supernatant is cleared by passage through a 0.45  $\mu$ m filter. The cleared lysate is applied to the monoclonal antibody array described above and allowed to incubate for 2 hours at 30°C. After binding the array is washed extensively as in Example 6, above. The location and amount of bound proteins are determined by optical detection.

All documents cited in the above specification are herein incorporated by reference. In addition, the copending U.S. patent application "Arrays of Proteins and Methods of Use Thereof", filed on July 14, 1999, with the identifier 24406-0004 P1, for the inventors Peter Wagner, Dana Ault-Riche, Steffen Nock, and Christian Itin, is herein incorporated by reference in its entirety. Various modifications and variations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the following claims.

What is claimed is:

1. An array of protein-capture agents, comprising:
  - (a) a substrate;
  - (b) at least one organic thinfilm covering some or all of the surface of the substrate; and
  - (c) a plurality of patches arranged in discrete, known regions on the portions of the substrate surface covered by organic thinfilm, wherein:
    - (i) each patch comprises protein-capture agents immobilized on the organic thinfilm, wherein said protein-capture agents of a given patch are capable of binding a particular expression product, or a fragment thereof, of a cell or population of cells in an organism; and
    - (ii) said array comprises a plurality of different protein-capture agents, each of which is capable of binding a different expression product, or fragment thereof, of the cell or population of cells.
2. The array of Claim 1 which comprises at least about 10 of said patches.
3. The array of Claim 2 which comprises at least about 100 of said patches.
4. The array of Claim 3 which comprises at least about  $10^3$  of said patches.
5. The array of Claim 1 which comprises at least about 10 different protein-capture agents.
6. The array of Claim 5 which comprises at least about 100 different protein-capture agents.

7. The array of Claim 6 which comprises at least about 1000 different protein-capture agents.
8. The array of Claim 1, wherein the area of the substrate surface covered by each of the patches is no more than about  $0.25 \text{ mm}^2$ .
9. The array of Claim 8, wherein the area of the substrate surface covered by each of the patches is between about  $1 \text{ }\mu\text{m}^2$  and about  $10,000 \text{ }\mu\text{m}^2$ .
10. The array of Claim 1, wherein the patches are all contained within an area of about  $1 \text{ cm}^2$  or less on the surface of the substrate.
11. The array of Claim 1, wherein the protein-capture agents are proteins.
12. The array of Claim 11, wherein the protein-capture agents are antibodies or antibody fragments.
13. The array of Claim 12, wherein the antibodies or antibody fragments have been derived by selection from a library using the phage display method.
14. The array of Claim 13, wherein the antibodies or antibody fragments have been derived by affinity binding to the proteins of a cellular extract or body fluid.
15. The array of Claim 12, wherein said antibodies or antibody fragments are selected from the group consisting of monoclonal antibodies, Fab fragments, and single-chain Fvs.
16. The array of Claim 1, wherein the organic thinfilm on the array is less than about 20 nm thick.



(b) detecting, either directly or indirectly, for the presence or amount of protein bound to each patch of the array.

30. A method of assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism, comprising:

(a) delivering the sample to an array of Claim 1 under conditions suitable for protein binding, wherein each of the proteins being assayed is a binding partner of the protein-capture agent of at least one patch on the array; and

(b) detecting, either directly or indirectly, for the presence or amount of protein bound to each patch of the array.

31. The method of claim 30, further comprising the step:  
further characterizing the proteins bound to at least one patch of the array.

32. The method of Claim 31, wherein said step of further characterizing the proteins comprises measuring the activity of the proteins.

33. A method for determining the protein expression pattern of a cell or a population of cells in an organism, comprising:

(a) delivering a sample containing the expression products, or fragments thereof, of the cell or population of cells to an array of Claim 1 under conditions suitable for protein binding; and

(b) detecting, either directly or indirectly, for the amount of protein bound to each patch of the array.

34. A method of comparing the protein expression patterns of two cells or populations of cells, comprising:

- (a) delivering a sample containing the expression products, or fragments thereof, of a first cell or population of cells to a first array of Claim 1 under conditions suitable for protein binding;
- (b) delivering a sample containing the expression products, or fragments thereof, of a second cell or population of cells to a second array, wherein the second array is identical to the first array;
- (c) detecting, either directly or indirectly, for the amount of protein bound to each patch on the washed first and second arrays; and
- (d) comparing the amounts of protein bound to the patches of the first array to the amounts of protein bound to the corresponding patches of the second array.

35. A method of evaluating a disease condition in a tissue in an organism, comprising:

- (a) contacting a sample comprising the expression products, or fragments thereof, of the cells of the tissue being evaluated with an array of Claim 1 under conditions suitable for protein binding, wherein the binding partners of a plurality of protein-capture agents on the array include proteins which are expression products, or fragments thereof, of the cells of the tissue and whose expression levels are indicative of the disease condition; and
- (b) detecting, directly or indirectly, for the amount of protein bound to each patch of the array.

36. A method for producing the array of Claim 1, comprising:

- (a) selecting recombinant bacteriophage expressing antibody fragments from a phage display library, wherein said recombinant bacteriophage are selected by affinity binding to a protein which is an expression product, or fragment thereof, of a cell or population of cells in an organism;
- (b) producing at least one purified sample of an antibody fragment from a bacteriophage selected in step (a); and

(c) repeating steps (a)-(b) with a different proteins which are expression products, or fragments thereof, of a cell or population of cells from the organism, or a fragment of the second protein, until the desired plurality of purified samples of different antibody fragments with different binding pairs is produced; and

(d) immobilizing the antibody fragment of each different purified sample onto an organic thinfilm on a separate patch on the surface of a substrate to form a plurality of patches of antibody fragments on discrete, known regions of the substrate surface.

37. A method for producing an array of protein-capture agents, comprising:

(a) selecting protein-capture agents from a library of protein-capture agents, wherein the protein-capture agents are selected by their binding affinity to the proteins in a cellular extract or body fluid;

(b) producing a plurality of purified samples of the selected protein-capture agents of step (a); and

(c) immobilizing the protein-capture agent of each different purified sample onto an organic thinfilm on a separate patch on the surface of a substrate to form a plurality of patches of protein-capture agents on discrete, known regions of the substrate surface.

38. The method of Claim 37, wherein said protein-capture agents are antibody fragments displayed on the surface of recombinant bacteriophages and said library of protein-capture agents is a phage display library.

39. A method of Claim 38, further comprising:

biasing the library of protein-capture agents by eliminating from the library those protein-capture agents which bind certain proteins, wherein the protein-capture agents which are eliminated are removed from the library by their binding affinity to said certain proteins.

40. The method of claim 39, wherein said certain proteins are proteins in a second cellular extract or body fluid.
41. An array of protein-capture agents produced by the method of Claim 37.
42. A method for producing an array of protein-capture agents, comprising:
- (a) selecting protein-capture agents from a library of protein-capture agents, wherein the protein-capture agents are selected by their binding affinity to proteins which are the expression products, or fragments thereof, of a cDNA expression library;
  - (b) producing a plurality of purified samples of the protein-capture agents of step (a); and
  - (c) immobilizing the protein-capture agent of each different purified sample onto an organic thinfilm on a separate patch on the surface of a substrate to form a plurality of patches of protein-capture agents on discrete, known regions of the surface of the substrate.
43. The method of Claim 42, wherein said protein-capture agents are antibody fragments displayed on the surface of recombinant bacteriophages and said library of protein-capture agents is a phage display library.
44. A method of Claim 42, further comprising:
- biasing the library of protein-capture agents by eliminating from the library those protein-capture agents which bind certain proteins, wherein the protein-capture agents which are eliminated are removed from the library by their binding affinity to said certain proteins.
45. An array of protein-capture agents produced by the method of Claim 42.

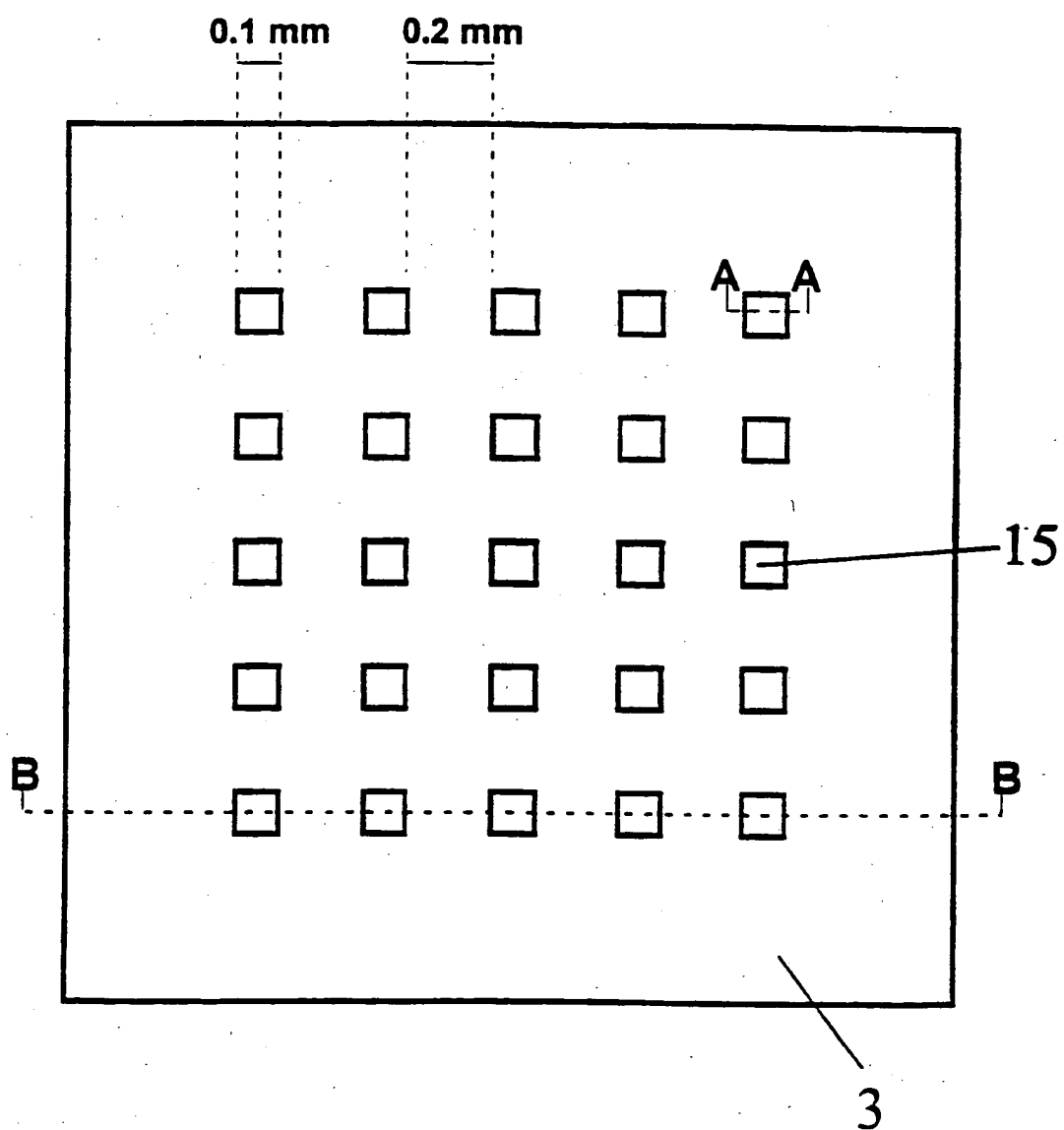


Fig. 1

A - A:

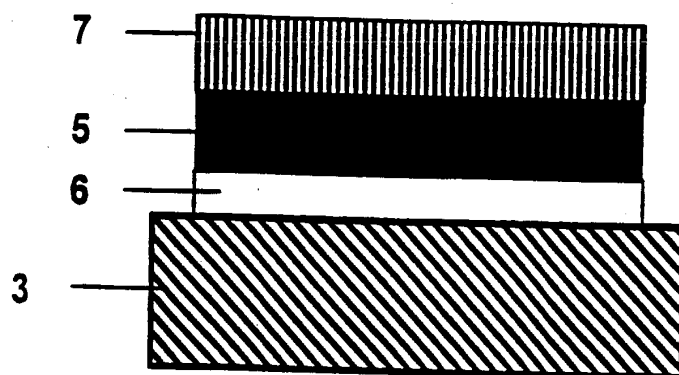


Fig. 2

**B - B :**

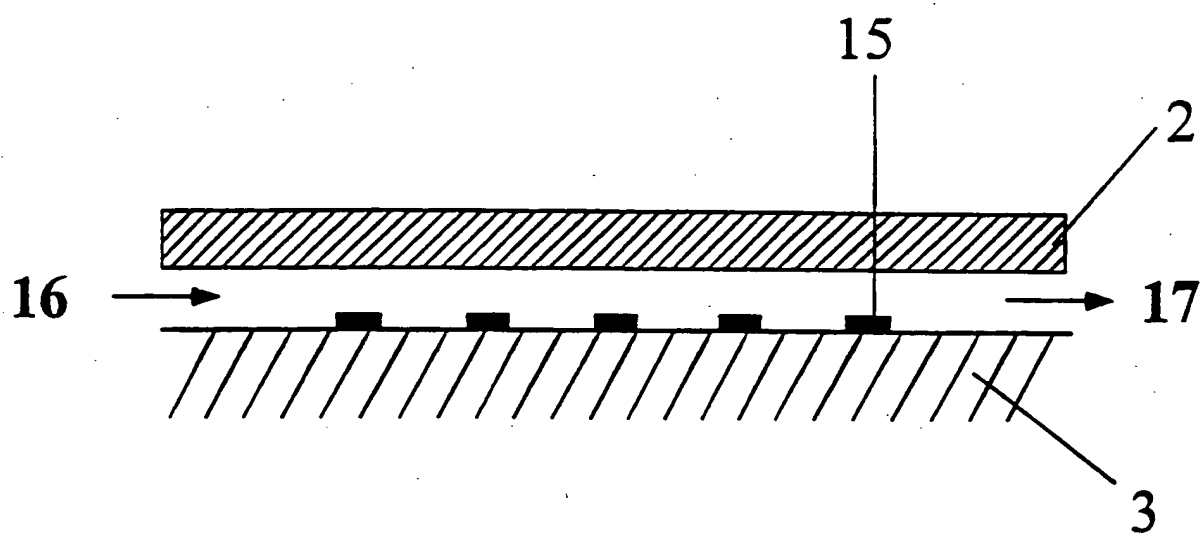


Fig. 3

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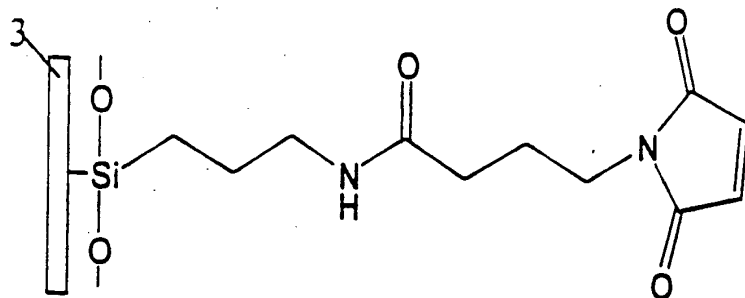


Fig. 4

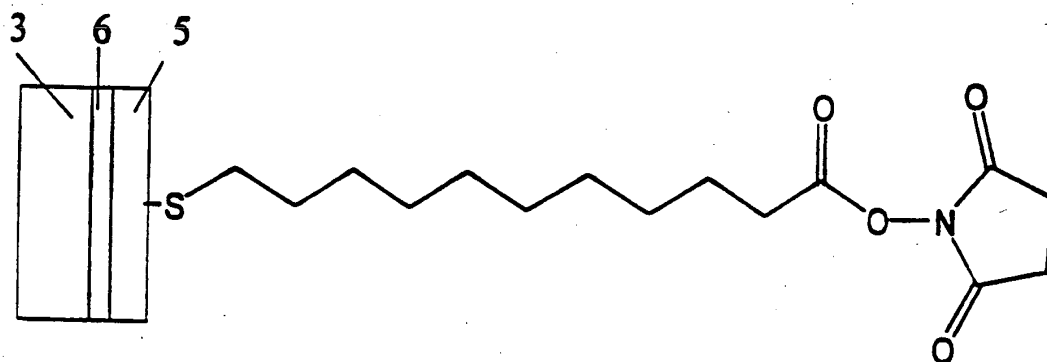


Fig. 5



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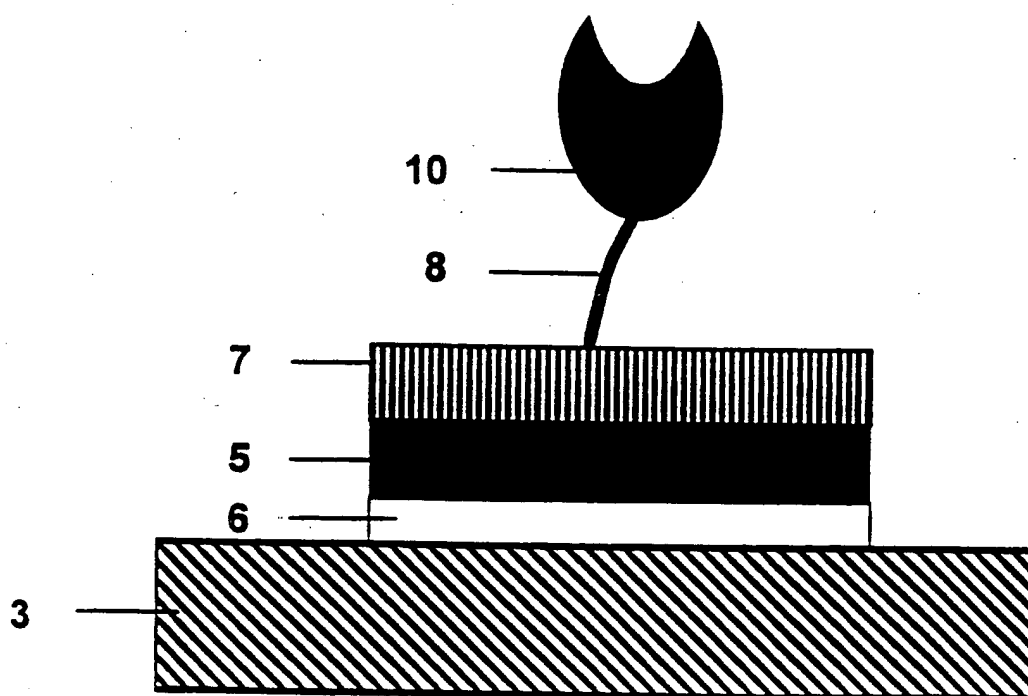


Fig. 6

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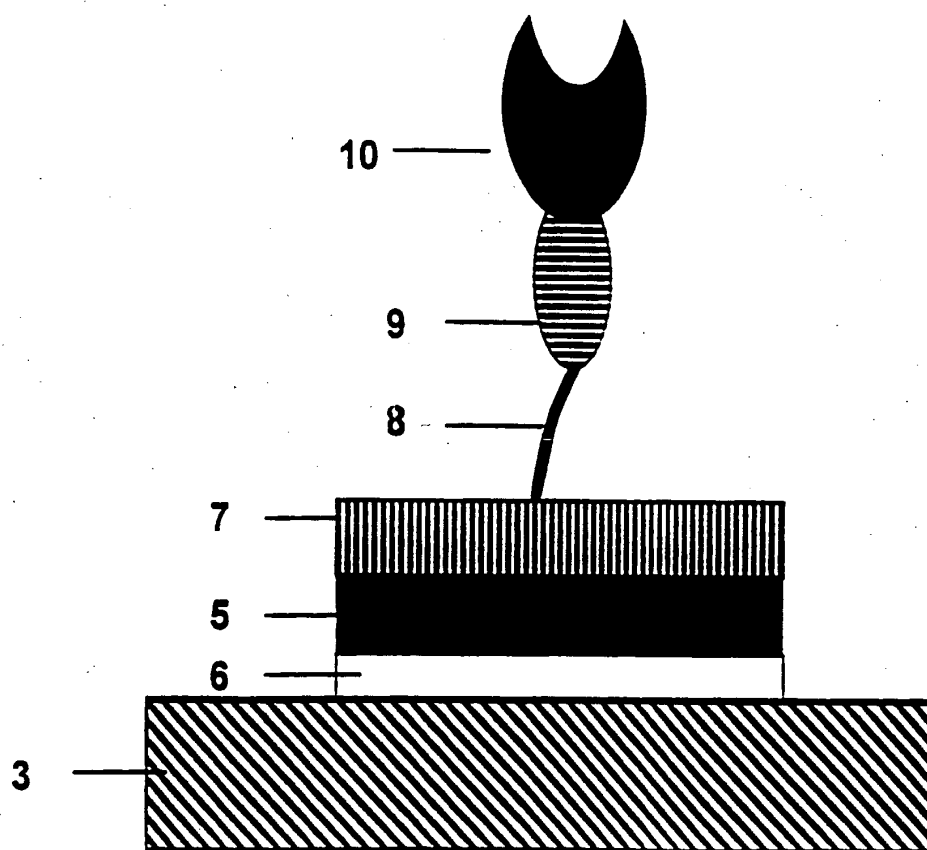


Fig. 7

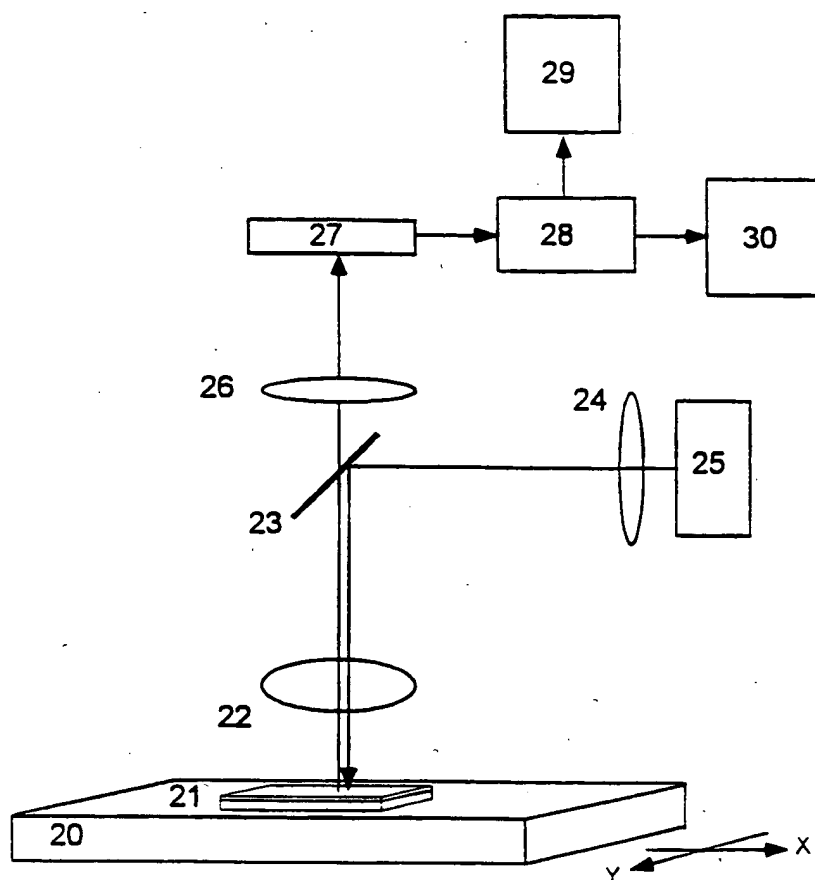


Fig. 8

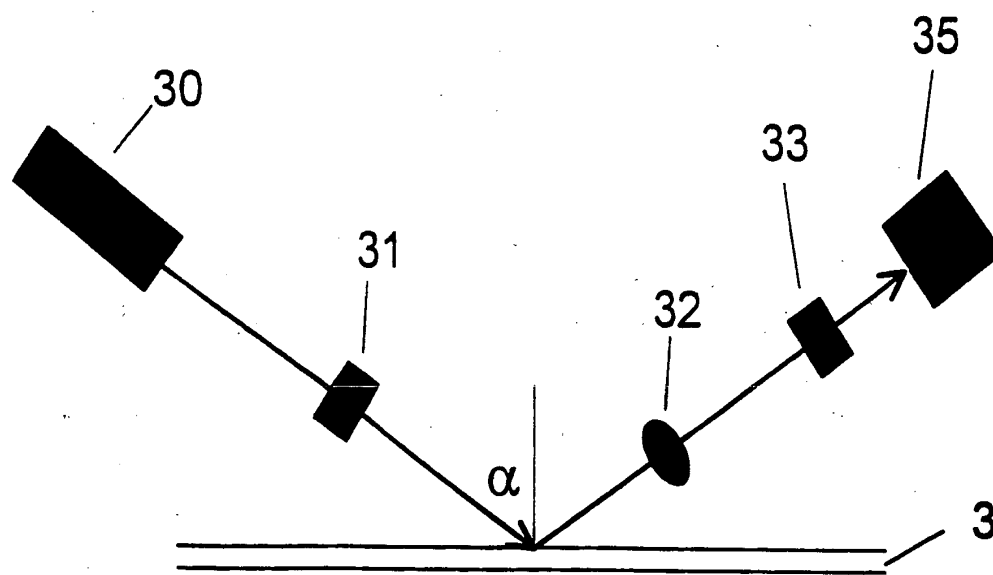


Fig. 9

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US99/15969 <b>(22) International Filing Date:</b> 14 July 1999 (14.07.99)  <b>(30) Priority Data:</b> 09/115,397 14 July 1998 (14.07.98) US  <b>(71) Applicant:</b> ZYOMYX, INC. [US/US]; 3912 Trust Way, Hayward, CA 94545 (US).  <b>(72) Inventors:</b> WAGNER, Peter; 2211 Village Court #7, Belmont, CA 94002 (US). AULT-RICHE, Dana; 972 Cajon Way, Palo Alto, CA 94303 (US). NOCK, Steffen; 3629 Glenwood Avenue, Redwood City, CA 94062 (US). ITIN, Christian; 315 Waverley Street #3, Menlo Park, CA 94025 (US).  <b>(74) Agents:</b> CHOW, Y., Ping et al.; Heller Ehrman White & McAuliffe, 525 University Avenue, Palo Alto, CA 94301-1900 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> MICRODEVICES FOR SCREENING BIOMOLECULES		
<b>(57) Abstract</b>  Methods and devices for the parallel, <i>in vitro</i> screening of biomolecular activity using miniaturized microfabricated devices are provided. The biomolecules immobilized on the surface of the devices of the present invention include proteins, polypeptides, polynucleotides, polysaccharides, phospholipids, and related unnatural polymers of biological relevance. These devices are useful in drug development, functional proteomics and clinical diagnostics and are preferably used for the parallel screening of families of related proteins.		

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## MICRODEVICES FOR SCREENING BIOMOLECULES

### BACKGROUND OF THE INVENTION

#### a) Field of the Invention

The present invention relates generally to microdevices and methods of using those devices for the parallel, *in vitro* screening of a plurality of biomolecule-analyte interactions. More specifically, the present invention relates to use of the devices for drug development, functional proteomics, and clinical diagnostics.

#### b) Description of Related Art

A vast number of new drug targets are now being identified using a combination of genomics, bioinformatics, genetics, and high-throughput biochemistry. Genomics provides information on the genetic composition and the activity of an organism's genes. Bioinformatics uses computer algorithms to recognize and predict structural patterns in DNA and proteins, defining families of related genes and proteins. The information gained from the combination of these approaches is expected to greatly boost the number of drug targets (usually, proteins).

The number of chemical compounds available for screening as potential drugs is also growing dramatically due to recent advances in combinatorial chemistry, the production of large numbers of organic compounds through rapid parallel and automated synthesis. The compounds produced in the combinatorial libraries being generated will far outnumber those compounds being prepared by traditional, manual means, natural product extracts, or those in the historical compound files of large pharmaceutical companies.

Both the rapid increase of new drug targets and the availability of vast libraries of chemical compounds creates an enormous demand for new technologies which improve the screening process. Current technological approaches which attempt to address this need include multiwell-plate based

screening systems, cell-based screening systems, microfluidics-based screening systems, and screening of soluble targets against solid-phase synthesized drug components.

Automated multiwell formats are the best developed high-throughput screening systems. Automated 96-well plate-based screening systems are the most widely used. The current trend in plate based screening systems is to reduce the volume of the reaction wells further, thereby increasing the density of the wells per plate (96-well to 384- and 1536-well per plate). The reduction in reaction volumes results in increased throughput, dramatically decreased bioreagent costs, and a decrease in the number of plates which need to be managed by automation.

However, although increases in well numbers per plate are desirable for high throughput efficiency, the use of volumes smaller than 1 microliter in the well format generates significant problems with evaporation, dispensing times, protein inactivation, and assay adaptation. Proteins are very sensitive to the physical and chemical properties of the reaction chamber surfaces. Proteins are prone to denaturation at the liquid/solid and liquid/air interfaces. Miniaturization of assays to volumes smaller than 1 microliter increases the surface to volume ratio substantially. (Changing volumes from 1 microliter to 10 nanoliter increases the surface ratio by 460%, leading to increased protein inactivation.) Furthermore, solutions of submicroliter volumes evaporate rapidly, within seconds to a few minutes, when in contact with air. Maintaining microscopic volumes in open systems is therefore very difficult.

Other types of high-throughput assays, such as miniaturized cell-based assays are also being developed. Miniaturized cell-based assays have the potential to generate screening data of superior quality and accuracy, due to their *in vivo* nature. However, the interaction of drug compounds with proteins other than the desired targets is a serious problem related to this approach which leads to a high rate of false positive results.

Microfluidics-based screening systems that measure *in vitro* reactions in solution make use of ten to several-hundred micrometer wide channels.

Micropumps, electroosmotic flow, integrated valves and mixing devices control liquid movement through the channel network. Microfluidic networks prevent evaporation but, due to the large surface to volume ratio, result in significant protein inactivation. The successful use of microfluidic networks in biomolecule screening remains to be shown.

Drug screening of soluble targets against solid-phase synthesized drug components is intrinsically limited. The surfaces required for solid state organic synthesis are chemically diverse and often cause the inactivation or non-specific binding of proteins, leading to a high rate of false-positive results. Furthermore, the chemical diversity of drug compounds is limited by the combinatorial synthesis approach that is used to generate the compounds at the interface. Another major disadvantage of this approach stems from the limited accessibility of the binding site of the soluble target protein to the immobilized drug candidates.

Miniaturized DNA chip technologies have been developed (for example, see U.S. Patent Nos. 5,412,087, 5,445, 934 and 5,744,305) and are currently being exploited for nucleic acid hybridization assays. However, DNA biochip technology is not transferable to protein assays because the chemistries and materials used for DNA biochips are not readily transferable to use with proteins. Nucleic acids withstand temperatures up to 100°C, can be dried and re-hydrated without loss of activity, and can be bound directly to organic adhesion layers supported by materials such as glass while maintaining their activity. In contrast, proteins must remain hydrated, kept at ambient temperatures, and are very sensitive to the physical and chemical properties of the support materials. Therefore, maintaining protein activity at the liquid-solid interface requires entirely different immobilization strategies than those used for nucleic acids. Additionally, the proper orientation of the protein at the interface is desirable to ensure accessibility of their active sites with interacting molecules. With miniaturization of the chip and decreased feature sizes the ratio of accessible to non-accessible antibodies becomes increasingly relevant.

In addition to the goal of achieving high-throughput screening of compounds against targets to identify potential drug leads, researchers also need to be able to identify highly specific lead compounds early in the drug discovery process. Analyzing a multitude of members of a protein family or forms of a polymorphic protein in parallel (multitarget screening) enables quick identification of highly specific lead compounds. Proteins within a structural family share similar binding sites and catalytic mechanisms. Often, a compound that effectively interferes with the activity of one family member also interferes with other members of the same family. Using standard technology to discover such additional interactions requires a tremendous effort in time and costs and as a consequence is simply not done.

However, cross-reactivity of a drug with related proteins can be the cause of low efficacy or even side effects in patients. For instance, AZT, a major treatment for AIDS, blocks not only viral polymerases, but also human polymerases, causing deleterious side effects. Cross-reactivity with closely related proteins is also a problem with nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin. These drugs inhibit cyclooxygenase-2, an enzyme which promotes pain and inflammation. However, the same drugs also strongly inhibit a related enzyme, cyclooxygenase-1, that is responsible for keeping the stomach lining and kidneys healthy, leading to common side-effects including stomach irritation.

The miniaturized, parallel screening of a plurality of protein interactions is also useful and necessary for a number of applications beyond high-throughput drug screening. For instance, the function of newly discovered proteins could be assayed effectively in a parallel format with a plurality of potential ligands or potential substrates of known protein families. Also, miniaturized diagnostic devices which allow for the analysis of a plurality of analytes by binding the analytes to proteins such as antibodies would be desirable.

For the foregoing reasons, there is a need for a miniaturized device and methods of using the device for the parallel, *in vitro*, screening of a plurality of

biomolecular interactions, especially the interactions of proteins with analytes or other proteins.

### SUMMARY OF THE INVENTION

The present invention is directed to a device and methods of use of the device that satisfy the need for the parallel, *in vitro*, screening of a plurality of biomolecular interactions, especially the interactions of proteins with analytes or other proteins.

One embodiment of the present invention provides a device for analyzing components of a fluid sample, comprising a plurality of noncontiguous reactive sites. Each of the reactive sites comprises a substrate, an organic thinfilm chemisorbed or physisorbed on a portion of a surface of the substrate, and a biological moiety immobilized on the organic thinfilm, wherein each of the reactive sites may independently react with a component of the fluid sample and are separated from each other by a region of the substrate that is free of organic thinfilm.

In a particularly preferred embodiment of the device, each of the reactive sites on the device of the invention is in a microchannel oriented parallel to microchannels of other reactive sites on the device, where the microchannels are microfabricated into or onto the substrate.

An alternative embodiment of the invention provides a device for analyzing components of a fluid sample that comprises a substrate, a plurality of parallel microchannels microfabricated into or onto said substrate, and a biological moiety immobilized within at least one of the parallel microchannels in such a way that the biological moiety may interact with a component of the fluid sample. In a preferred embodiment, the biological moiety is a protein.

Methods of using the devices of the invention are also provided by the present invention. In one embodiment, the invention provides for a method of screening a plurality of biological moieties in parallel for their ability to interact with a component of a fluid sample. This method comprises first delivering the

fluid sample to the reactive sites of the invention device, where each of the different biological moieties is immobilized on a different reactive site of the device and detecting, either directly or indirectly, for the interaction of the component with the immobilized biological moiety at each reactive site. The interaction being assayed may be a binding interaction, catalysis, or translocation by a membrane protein through a lipid bilayer.

In an alternative embodiment of the invention, the device of the invention is used to screen a plurality of components, each in separate fluid samples, for their ability to interact with a biological moiety. The method of this embodiment comprises first delivering each of the different fluid samples to separate reactive sites of the invention device, wherein the separate reactive sites of the device each comprise the immobilized biological moiety. The next step comprises detecting, either directly or indirectly, for the interaction of the immobilized biological moiety at each reactive site with the component delivered to that reactive site. Again, the interaction being assayed may be a binding interaction, catalysis, or translocation by a membrane protein through a lipid bilayer.

In another embodiment of the present invention, a similar method is used to screen a fluid sample for the presence or amount of a plurality of analytes (in parallel). This method has potential applications in diagnostics. The method comprises delivering the fluid sample to a plurality of reactive sites on the invention device, wherein each of the reactive sites comprises an immobilized biological moiety which can either react, bind, or otherwise interact with at one of said plurality of analytes. The method also comprises a final step of detecting for the interaction of the analyte with the immobilized biological moiety of each reactive site.

In another embodiment of the invention, the device may also be used to screen a plurality of binding candidates in parallel for their ability to bind to a biological moiety. In the method of this embodiment, different fluid samples, each containing a different binding candidate (or a different mixture of binding candidates) to be tested, are delivered separate reactive sites of the invention

device, wherein the separate reactive sites each comprise the immobilized biological moiety. The next step of the method comprises detecting, either directly or indirectly, for the presence or amount of the binding candidate.

The present invention also provides for methods of determining in parallel whether or not each of a plurality of proteins belong to a certain protein family based on either binding to a common ligand or reactivity with a common substrate. These methods involve delivering a fluid sample comprising a ligand or substrate of a known protein family to the reactive sites of the invention device which each contain one of the different proteins to be assayed and then detecting, either directly or indirectly, for binding or reaction with the known ligand that is characteristic of the protein family.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the top view of a covered microchannel array device.

Figure 2 shows a cross section of a covered microchannel array fabricated by bulk micromachining.

Figure 3 shows a cross section of a covered microchannel array fabricated by sacrificial micromachining.

Figure 4 shows a thiolreactive monolayer on a substrate.

Figure 5 shows an aminoreactive monolayer on a coated substrate.

Figure 6 shows a biological moiety immobilized on a monolayer-coated substrate via an affinity tag.

Figure 7 shows a cross section view of a biomolecule-coated microchannel in a microchannel array device.

Figure 8 shows a biological moiety immobilized on a monolayer-coated substrate via an affinity tag and an adaptor molecule.

Figure 9 shows a schematic diagram of a fluorescence detection unit which may be used to monitor interaction of the immobilized biological moieties of a microchannel array with an analyte.

## DETAILED DESCRIPTION OF THE INVENTION

A variety of devices and methods useful for drug development, proteomics, and, clinical diagnostics are provided by the present invention.

## (a) Definitions

The terms "biological moiety" and "biomolecule" are used interchangeably and each refer to any entity that either has, or is suspected of having, a physiological function. The biological moiety may be a single molecule or may be a macromolecular complex. One example of a biological moiety is a polynucleotide. A preferred biological moiety is a protein. The protein may be any intracellular or an extracellular protein, including any membrane protein or secreted protein. Other possible biological moieties include small molecule compounds which can act as inhibitors of enzymes or which can bind other biomolecules. For instance, a biological moiety may optionally be a protein-capture agent.

The term "polynucleotide" means a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a manner similar to naturally occurring nucleotides. The polynucleotide may be obtained from a natural source or produced *in vitro* or *in vivo* by enzymatic or chemical synthesis. No distinction is made herein between a nucleic acid, a polynucleotide, and an oligonucleotide. Preferably the polynucleotide comprises at least about 16 nucleotides.

A "protein" means a polymer of amino acid residues linked together by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. Typically, however, a protein will be at least about six amino acids long. Preferably, if the protein is a short peptide, it will be at least about 10 amino acid residues long. A protein may be naturally occurring, recombinant, or synthetic, or any combination of these. A protein may also be just a fragment of a naturally occurring protein or peptide. A protein may



be a single molecule or may be a multi-molecular complex. The term protein may also apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid.

An amino acid polymer in which one or more amino acid residues is an "unnatural" amino acid, not corresponding to any naturally occurring amino acid, is also encompassed by the use of the term "protein" herein.

A "fragment of a protein" means a protein which is a portion of another protein. For instance, fragments of a proteins may be polypeptides obtained by doing a digest of full-length protein isolated from cultured cells. A fragment of a protein will typically comprise at least six amino acids. More typically, the fragment will comprise at least ten amino acids. Preferably, the fragment comprises at least about 16 amino acids.

The term "antibody" means an immunoglobulin, whether natural or wholly or partially synthetically produced. All derivatives thereof which maintain specific binding ability are also included in the term. The term also covers any protein having a binding domain which is homologous or largely homologous to an immunoglobulin binding domain, including chimeric and humanized antibodies. These proteins may be derived from natural sources, or partly or wholly synthetically produced. An antibody may be monoclonal or polyclonal. The antibody may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgA, IgD, and IgE. Derivatives of the IgG class, however, are preferred in the present invention.

The term "antibody fragment" refers to any derivative of an antibody which is less than full-length. Preferably, the antibody fragment retains at least a significant portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')<sub>2</sub>, scFv, Fv, dsFv diabody, and Fd fragments. The antibody fragment may be produced by any means. For instance, the antibody fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or it may be recombinantly produced from a gene encoding the partial antibody sequence. Alternatively, the

antibody fragment may be wholly or partially synthetically produced. The antibody fragment may optionally be a single chain antibody fragment. Alternatively, the fragment may comprise multiple chains which are linked together, for instance, by disulfide linkages. The fragment may also optionally be a multimolecular complex. A functional antibody fragment will typically comprise at least about 50 amino acids and more typically will comprise at least about 200 amino acids.

Single-chain Fvs (scFvs) are recombinant antibody fragments consisting of only the variable light chain ( $V_L$ ) and variable heavy chain ( $V_H$ ) covalently connected to one another by a polypeptide linker. Either  $V_L$  or  $V_H$  may be the  $NH_2$ -terminal domain. The polypeptide linker may be of variable length and composition so long as the two variable domains are bridged without serious steric interference. Typically, the linkers are comprised primarily of stretches of glycine and serine residues with some glutamic acid or lysine residues interspersed for solubility.

"Diabodies" are dimeric scFvs. The components of diabodies typically have shorter peptide linkers than most scFvs and they show a preference for associating as dimers.

An "Fv" fragment is an antibody fragment which consists of one  $V_H$  and one  $V_L$  domain held together by noncovalent interactions. The term "dsFv" is used herein to refer to an Fv with an engineered intermolecular disulfide bond to stabilize the  $V_H$ - $V_L$  pair.

A " $F(ab')_2$ " fragment is an antibody fragment essentially equivalent to that obtained from immunoglobulins (typically IgG) by digestion with an enzyme pepsin at pH 4.0-4.5. The fragment may be recombinantly produced.

A "Fab" fragment is an antibody fragment essentially equivalent to that obtained by reduction of the disulfide bridge or bridges joining the two heavy chain pieces in the  $F(ab')_2$  fragment. The Fab' fragment may be recombinantly produced.

A "Fab" fragment is an antibody fragment essentially equivalent to that obtained by digestion of immunoglobulins (typically IgG) with the enzyme papain. The Fab fragment may be recombinantly produced. The heavy chain segment of the Fab fragment is the Fd piece.

The term "protein-capture agent" means a molecule or a multi-molecular complex which can bind a protein to itself. Protein-capture agents preferably bind their binding partners in a substantially specific manner. Protein-capture agents with a dissociation constant ( $K_D$ ) of less than about  $10^{-6}$  are preferred. Antibodies or antibody fragments are highly suitable as protein-capture agents. Antigens may also serve as protein-capture agents, since they are capable of binding antibodies. A receptor which binds a protein ligand is another example of a possible protein-capture agent. Protein-capture agents are understood not to be limited to agents which only interact with their binding partners through noncovalent interactions. Protein-capture agents may also optionally become covalently attached to the proteins which they bind. For instance, the protein-capture agent may be photocrosslinked to its binding partner following binding.

The term "binding partner" means a protein which is bound by a particular protein-capture agent, preferably in a substantially specific manner. In some cases, the binding partner may be the protein normally bound *in vivo* by a protein which is a protein-capture agent. In other embodiments, however, the binding partner may be the protein or peptide on which the protein-capture agent was selected (through *in vitro* or *in vivo* selection) or raised (as in the case of antibodies). A binding partner may be shared by more than one protein-capture agent. For instance, a binding partner which is bound by a variety of polyclonal antibodies may bear a number of different epitopes. One protein-capture agent may also bind to a multitude of binding partners (for instance, if the binding partners share the same epitope),

"Conditions suitable for protein binding" means those conditions (in terms of salt concentration, pH, detergent, protein concentration, temperature, etc.) which allow for binding to occur between a protein-capture agent and its binding

partner in solution. Preferably, the conditions are not so lenient that a significant amount of nonspecific protein binding occurs.

A "body fluid" may be any liquid substance extracted, excreted, or secreted from an organism or tissue of an organism. The body fluid need not necessarily contain cells. Body fluids of relevance to the present invention include, but are not limited to, whole blood, serum, urine, plasma, cerebral spinal fluid, tears, sinovial fluid, and amniotic fluid.

The term "substrate" refers to the bulk, underlying, and core material of the devices of the invention.

The terms "micromachining" and "microfabrication" both refer to any number of techniques which are useful in the generation of microstructures (structures with feature sizes of sub-millimeter scale). Such technologies include, but are not limited to, laser ablation, electrodeposition, physical and chemical vapor deposition, photolithography, and wet chemical and dry etching. Related technologies such as injection molding and LIGA (x-ray lithography, electrodeposition, and molding) are also included. Most of these techniques were originally developed for use in semiconductors, microelectronics, and Micro-ElectroMechanical Systems (MEMS) but are applicable to the present invention as well.

The term "coating" means a layer that is either naturally or synthetically formed on or applied to the surface of the substrate. For instance, exposure of a substrate, such as silicon, to air results in oxidation of the exposed surface. In the case of a substrate made of silicon, a silicon oxide coating is formed on the surface upon exposure to air. In other instances, the coating is not derived from the substrate and may be placed upon the surface via mechanical, physical, electrical, or chemical means. An example of this type of coating would be a metal coating that is applied to a silicon or polymer substrate or a silicon nitride coating that is applied to a silicon substrate. Although a coating may be of any thickness, typically the coating has a thickness smaller than that of the substrate.

An "interlayer" is an additional coating or layer that is positioned between the first coating and the substrate. Multiple interlayers may optionally be used together. The primary purpose of a typical interlayer is to aid adhesion between the first coating and the substrate. One such example is the use of a titanium or chromium interlayer to help adhere a gold coating to a silicon or glass surface. However, other possible functions of an interlayer are also anticipated. For instance, some interlayers may perform a role in the detection system of the device (such as a semiconductor or metal layer between a nonconductive substrate and a nonconductive coating).

An "organic thinfilm" is a thin layer of organic molecules which has been applied to a substrate or to a coating on a substrate if present. Typically, an organic thinfilm is less than about 20 nm thick. Optionally, an organic thinfilm may be less than about 10 nm thick. An organic thinfilm may be disordered or ordered. For instance, an organic thinfilm can be amorphous (such as a chemisorbed or spin-coated polymer) or highly organized (such as a Langmuir-Blodgett film or self-assembled monolayer). An organic thinfilm may be heterogeneous or homogeneous. Organic thinfilms which are monolayers are preferred. A lipid bilayer or monolayer is a preferred organic thinfilm. Optionally, the organic thinfilm may comprise a combination of more than one form of organic thinfilm. For instance, an organic thinfilm may comprise a lipid bilayer on top of a self-assembled monolayer. A hydrogel may also compose an organic thinfilm. The organic thinfilm will typically have functionalities exposed on its surface which serve to enhance the surface conditions of a substrate or the coating on a substrate in any of a number of ways. For instance, exposed functionalities of the organic thinfilm are typically useful in the binding or covalent immobilization of the biological moieties to the device. Alternatively, the organic thinfilm may bear functional groups (such as polyethylene glycol (PEG)) which reduce the non-specific binding of biomolecules and other analytes to the surface. Other exposed functionalities serve to tether the thinfilm to the surface of the substrate or the coating. Particular functionalities of the organic

thinfilm may also be designed to enable certain detection techniques to be used with the surface. Alternatively, the organic thinfilm may serve the purpose of preventing inactivation of a biological moiety immobilized on the device from occurring upon contact with the surface of a substrate or a coating on the surface of a substrate.

A "monolayer" is a single-molecule thick organic thinfilm. A monolayer may be disordered or ordered. A monolayer may optionally be a polymeric compound, such as a polynonionic polymer, a polyionic polymer, or a block-copolymer. For instance, the monolayer may be composed of a poly(amino acid) such as polylysine. A monolayer which is a self-assembled monolayer, however, is most preferred. One face of the self-assembled monolayer is typically composed of chemical functionalities on the termini of the organic molecules that are chemisorbed or physisorbed onto the surface of the substrate or the coating, if present, on the substrate. Examples of suitable functionalities of monolayers include the positively charged amino groups of poly-L-lysine for use on negatively charged surfaces and thiols for use on gold surfaces. Typically, the other face of the self-assembled monolayer is exposed and may bear any number of chemical functionalities (end groups). Preferably, the molecules of the self-assembled monolayer are highly ordered.

A "self-assembled monolayer" is a monolayer which is created by the spontaneous assembly of molecules. The self-assembled monolayer may be ordered, disordered, or exhibit short- to long-range order.

An "affinity tag" is a functional moiety capable of directly or indirectly immobilizing a biological moiety onto an exposed functionality of the organic thinfilm. Preferably, the affinity tag enables the site-specific immobilization and thus enhances orientation of the biological moiety onto the organic thinfilm. In some cases, the affinity tag may be a simple chemical functional group. Other possibilities include amino acids, poly(amino acid) tags, or full-length proteins. Still other possibilities include carbohydrates and nucleic acids. For instance, the affinity tag may be a polynucleotide which hybridizes to another polynucleotide

serving as a functional group on the organic thinfilm or another polynucleotide serving as an adaptor. The affinity tag may also be a synthetic chemical moiety. If the organic thinfilm of each of the sites comprises a lipid bilayer or monolayer, then a membrane anchor is a suitable affinity tag. The affinity tag may be covalently or noncovalently attached to the biological moiety. For instance, if the affinity tag is covalently attached to a biological moiety which is a protein, it may be attached via chemical conjugation or as a fusion protein. The affinity tag may also be attached to the biological moiety via a cleavable linkage. Alternatively, the affinity tag may not be directly in contact with the biological moiety. The affinity tag may instead be separated from the biological moiety by an adaptor. The affinity tag may immobilize the biological moiety to the organic thinfilm either through noncovalent interactions or through a covalent linkage.

An "adaptor", for purposes of this invention, is any entity that links an affinity tag to the immobilized biological moiety of the device. The adaptor may be, but need not necessarily be, a discrete molecule that is noncovalently attached to both the affinity tag and the biological moiety. The adaptor can instead be covalently attached to the affinity tag or the biological moiety or both (via chemical conjugation or as a fusion protein, for instance). Proteins such as full-length proteins, polypeptides, or peptides are typical adaptors. Other possible adaptors include carbohydrates and nucleic acids.

The term "fusion protein" refers to a protein composed of two or more polypeptides that, although typically unjoined in their native state, are joined by their respective amino and carboxyl termini through a peptide linkage to form a single continuous polypeptide. It is understood that the two or more polypeptide components can either be directly joined or indirectly joined through a peptide linker/spacer.

The term "normal physiological condition" means conditions that are typical inside a living organism or a cell. While it is recognized that some organs or organisms provide extreme conditions, the intra-organismal and intra-cellular environment normally varies around pH 7 (*i.e.*, from pH 6.5 to pH 7.5), contains

water as the predominant solvent, and exists at a temperature above 0°C and below 50°C. It will be recognized that the concentration of various salts depends on the organ, organism, cell, or cellular compartment used as a reference.

"Proteomics" means the study of or the characterization of either the proteome or some fraction of the proteome. The "proteome" is the total collection of the intracellular proteins of a cell or population of cells and the proteins secreted by the cell or population of cells. This characterization most typically includes measurements of the presence, and usually quantity, of the proteins which have been expressed by a cell. The function, structural characteristics (such as post translational modification), and location within the cell of the proteins may also be studied. "Functional proteomics" refers to the study of the functional characteristics, activity level, and structural characteristics of the protein expression products of a cell or population of cells.

(b) The devices of the invention.

In one aspect, the present invention provides a device for analyzing components of a fluid sample. This device comprises a plurality of noncontiguous reactive sites, each of which comprises the following: a substrate; an organic thinfilm chemisorbed or physisorbed on a portion of a surface of the substrate; and a biological moiety immobilized on the organic thinfilm, wherein each of the sites may independently react with a component of the fluid sample and are separated from each other by a region of the substrate that is free of the organic thinfilm.

In a preferred embodiment, the device comprises at least about 10 reactive sites. In an especially preferred embodiment, the device comprises at least about 100 reactive sites.

In a preferred embodiment of the present invention the device comprises a micromachined or microfabricated device. The device is optionally a microdevice with dimensions on the millimeter to centimeter scale.

In a preferred embodiment of the invention, each of the reactive sites of the device is in a microchannel oriented parallel to microchannels of other reactive



sites on the device. The microchannels of such a device have optionally been microfabricated or micromachined into or onto the substrate of the device. A reactive site may optionally cover the entire interior surface of the microchannel or alternatively, only a portion of the interior surface of the microchannel.

In another embodiment, the invention provides a device for analyzing components of a fluid sample which comprises a substrate, a plurality of parallel microchannels microfabricated into or onto the substrate, and a biological moiety immobilized within at least one of the parallel microchannels, wherein the biological moiety may interact with a component of the fluid sample. Preferably, a number of parallel microchannels will comprise immobilized biological moieties. It is also preferred that the immobilized biological moiety of each microchannel be immobilized on an organic thinfilm on at least a portion of the inner surface of the microchannel.

Figure 1 illustrates one embodiment of the invention showing an array of microchannels 1 that have been fabricated into a bulk substrate material. In the particular device shown, forty-eight parallel microchannels 1 have been microfabricated into a substrate 3. A glass cover 2 covers a portion of the microchannel array.

In one embodiment of the invention, the device comprises at least 2 parallel microchannel reactive sites. In another embodiment of the invention, the device comprises at least 10 parallel microchannel reactive sites. In a preferred embodiment of the invention, the device comprises at least 100 parallel microchannel reactive sites. In a particularly preferred embodiment, the device comprises from about 100 to about 500 parallel microchannels. The microchannels are typically separated from one another by from about 10  $\mu\text{m}$  to about 5 mm. The device may optionally comprise from about 2 to about 500 parallel microchannels per  $\text{cm}^2$  of substrate.

The dimensions of the microchannels may vary. However, in preferred embodiments the scale is small enough so as to only require minute fluid sample volumes. The width and depth of each microchannel of the invention device is

typically between about 10  $\mu\text{m}$  and about 500  $\mu\text{m}$ . In a preferred embodiment of the device, the width and depth of each microchannel is between about 50 and 200  $\mu\text{m}$ . The length of each microchannel is from about 1 to about 20 mm in length. In a preferred embodiment, the length of each microchannel is from about 2 to about 8 mm long. Any channel cross-section geometry (trapezoidal, rectangular, v-shaped, semicircular, etc.) may be employed in the device. The geometry is determined by the type of microfabrication or micromachining process used to generate the microchannels, as is known in the art. Trapezoidal or rectangular cross-section geometries are preferred for the microchannels, since they readily accommodate standard fluorescence detection methods.

Numerous different materials may be used as the substrate of the invention device. The substrate may be organic or inorganic, biological or non-biological, or any combination of these materials. The substrate can optionally be transparent or translucent. Substrates suitable for micromachining or microfabrication are preferred. The substrate of the invention can optionally comprise a material selected from a group consisting of silicon, silica, quartz, glass, controlled pore glass, carbon, alumina, titania, tantalum oxide, germanium, silicon nitride, zeolites, and gallium arsenide. Many metals such as gold, platinum, aluminum, copper, titanium, and their alloys are also options for substrates. In addition, many ceramics and polymers may also be used as substrates. Polymers which may be used as substrates include, but are not limited to, the following: polystyrene; poly(tetra)fluoroethylene (PTFE); polyvinylidenedifluoride; polycarbonate; polymethylmethacrylate; polyvinylethylene; polyethyleneimine; poly(etherether)ketone; polyoxymethylene (POM); polyvinylphenol; polylactides; polymethacrylimide (PMI); polyalkenesulfone (PAS); polypropylene; polyethylene; polyhydroxyethylmethacrylate (HEMA); polydimethylsiloxane; polyacrylamide; polyimide; and block-copolymers. Preferred substrates for the device include silicon, silica, glass, and polymers. The substrate may also be a combination of any of the aforementioned substrate materials.

In order to generate a plurality of reactive sites, such as a parallel array of microchannels, the substrate material first has to be cleaned to remove contaminants such as solvent stains, dust, or organic residues. A variety of cleaning procedures can be used depending on the substrate material and origin of contaminants. These include wet immersion processes (for example, RCA1+2, "pyranha", solvents), dry vapor phase cleaning, thermal treatment, plasma or glow discharge techniques, polishing with abrasive compounds, short-wavelength light exposure, ultrasonic agitation and treatment with supercritical fluids.

Channels can then be formed on the surface of the substrate by either (1) bulk micromachining, (2) sacrificial micromachining, (3) LIGA (high aspect ratio plating) or (4) other techniques, or any combination thereof. Such techniques are well known in the semiconductor and microelectronics industries and are described in, for example, Ghandi, *VLSI Fabrication Principles*, Wiley (1983) and Sze, *VLSI Technology, 2nd. Ed.*, McGraw-Hill (1988); Wolf and Taube, *Silicon Processing for the VLSI Era, Vol. 1*, Lattice Press (1986), and Madou, *Fundamentals of Microfabrication*, CRC Press (1997).

In bulk micromachining, large portions of the substrate are removed to form rectangular or v-shaped grooves comprising the final dimensions of the microchannels. This process is usually carried out with standard photolithographic techniques involving spin-coating of resist materials, illumination through lithography masks followed by wet-chemical development and posttreatment steps such as descumming and post-baking. The resulting resist pattern is then used as an etch resist material for subsequent wet or dry etching of the bulk material to form the desired topographical structures. Typical resist materials include positive and negative organic resists (such as Kodak 747, PR102), inorganic materials (such as polysilicon, silicon nitride) and biological etch resists (for example Langmuir-Blodgett films and two-dimensional protein crystals such as the S-layer of *Sulfolobus acidocladarius*). Pattern transfer into the substrate and resist stripping occurs via wet-chemical and dry etching

techniques including plasma etching, reactive ion etching, sputtering, ion-beam-assisted chemical etching and reactive ion beam etching.

In one embodiment of the invention, for instance, a photoresist may be spincoated onto a cleaned 4 inch Si(110) wafer. Ultraviolet light exposure through a photomask onto the photoresist then results in a pattern of channels in the photoresist, exposing a pattern of strips of the silicon underneath. Wet-chemical etching techniques can then be applied to etch the channel pattern into the silicon. Next, a thin layer of titanium can be coated on the surface. A thin layer of gold is then coated on the surface via thermal or electron beam evaporation. Standard resist stripping follows. (Alternatively, the gold-coating could be carried out after the strip resist.)

Figure 2 shows a cross section view of one example of a microchannel array fabricated by bulk micromachining. A microchannel 1 in substrate 3 is covered by a glass cover 2. At the bottom of the microchannel, the surface of the substrate 3 is covered with a coating 5, separated by an interlayer 6.

In sacrificial micromachining, the substrate is left essentially untouched. Various thick layers of other materials are built up by vapor deposition, plasma-enhanced chemical vapor deposition (PECVD) or spin coating and selectively remain behind or are removed by subsequent processing steps. Thus, the resulting channel walls are chemically different from the bottom of the channels and the resist material remains as part of the microdevice. Typical resist materials for sacrificial micromachining are silicon nitride ( $\text{Si}_3\text{N}_4$ ), polysilicon, thermally grown silicon oxide and organic resists such as SU-8 and polyimides allowing the formation of high aspect-ratio features with straight sidewalls.

Figure 3 shows a cross section view of one example of a microchannel array that has been fabricated by sacrificial micromachining. Microchannel 1 has walls that consist of photoresist 4 and a floor that comprises a substrate 3 that is covered with a coating 5 plus an interlayer 6. A glass cover 2 covers the microchannel 1.

In high-aspect ratio plating or LIGA, three-dimensional metal structures are made by high-energy X-ray radiation exposures on materials coated with X-ray resists. Subsequent electrodeposition and resist removal result in metal structures that can be used for precision plastic injection molding. These injection-molded plastic parts can be used either as the final microdevice or as lost molds. The LIGA process has been described by Becker et al., *Microelectron Engineering* (1986) 4:35-56 and Becker et al., *Naturwissenschaften* (1982) 69:520-523.

Alternative techniques for the fabrication of microchannel arrays include focused ion-beam (FIB) milling, electrostatic discharge machining (EDM), ultrasonic drilling, laser ablation (US Patent No. 5,571,410), mechanical milling and thermal molding techniques. One skilled in the art will recognize that many variations in microfabrication or micromachining techniques may be used to construct the device of the present invention.

In one embodiment, transparent or translucent covers are attached to the substrate via anodic bonding or adhesive coatings, resulting in microchannel arrays with inlet and outlet ports. In a preferred embodiment, the microchannel covers are glass, especially Pyrex or quartz glass. In alternative embodiments, a cover which is neither transparent nor translucent may be bonded or otherwise attached to the substrate to enclose the microchannels. In other embodiments the cover may be part of a detection system to monitor the interaction between biological moieties immobilized within the channel and an analyte. Alternatively, a polymeric cover may be attached to a polymeric substrate channel array by other means, such as by the application of heat with pressure or through solvent-based bonding.

One particular embodiment of a covered microchannel array is illustrated by Figure 1. In this device, a transparent glass cover 2 covers most of the length, although not all, of each of the parallel microchannels of the array. Since in this particular embodiment the microchannels do not extend fully to the edge of the substrate, the incomplete coverage of the channel length provides an inlet and outlet port for each of the microchannels.

Attachment of a cover to the microchannel array can precede formation of the organic thinfilm on the reactive sites. If this is the case, then the solution which contains the components of the organic thinfilm (typically an organic solvent) can be applied to the interior of the channels via microfabricated dispensing systems that have integrated microcapillaries and suitable entry/exit ports. Alternatively, the organic thinfilms can be deposited in the microchannels prior to enclosure of the microchannels. For these embodiments, organic thinfilms such as monolayers can optionally be transferred to the inner microchannel surfaces via simple immersion or through microcontact printing (see PCT Publication WO 96/29629). In a most preferred embodiment, the organic thinfilm in all of the microchannels is identical. In such a case, simple immersion of the microchannel array or incubation of all of the microchannel interiors with the same fluid containing the thinfilm components is sufficient.

The volume of each enclosed microchannel may optionally be from about 5 nanoliters to about 300 nanoliters. In a preferred embodiment, the volume of an enclosed microchannel of the invention device is between 10 and 50 nanoliters.

Volumes of fluid may be moved through each microchannel by a number of standard means known to those skilled in the art. The sophisticated means required for moving fluids through microfluidic devices and mixing in microtiter plates are not needed for the microchannel array of the present invention. Simple liquid exchange techniques commonly used with capillary technologies will suffice. For instance, fluid may be moved through the channel using standard pumps. Alternatively, more sophisticated methods of fluid movement through the microchannels such as electro-osmosis may be employed (for example, see US Patent No. 4,908,112).

In one embodiment of the present invention, bulk-loading dispensing devices can be used to load all microchannels of the device at once with the same fluid. Alternatively, integrated microcapillary dispensing devices may be microfabricated out of glass or other substrates to load fluids separately to each microchannel of the device.

After formation of a microchannel, the sides, bottom, or cover of the microchannel or any portion or combination thereof, can then be further chemically modified to achieve the desired bioreactive and biocompatible properties.

The reactive sites of the device may optionally further comprise a coating between a substrate and its organic thinfilm. This coating may either be formed on the substrate or applied to the substrate. The substrate can be modified with a coating by using thin-film technology based, for example, on physical vapor deposition (PVD), thermal processing, or plasma-enhanced chemical vapor deposition (PECVD). Alternatively, plasma exposure can be used to directly activate or alter the substrate and create a coating. For instance, plasma etch procedures can be used to oxidize a polymeric surface (*i.e.*, polystyrene or polyethylene to expose polar functionalities such as hydroxyls, carboxylic acids, aldehydes and the like).

The coating is optionally a metal film. Possible metal films include aluminum, chromium, titanium, tantalum, nickel, stainless steel, zinc, lead, iron, copper, magnesium, manganese, cadmium, tungsten, cobalt, and alloys or oxides thereof. In a preferred embodiment, the metal film is a noble metal film. Noble metals that may be used for a coating include, but are not limited to, gold, platinum, silver, and copper. In an especially preferred embodiment, the coating comprises gold or a gold alloy. Electron-beam evaporation may be used to provide a thin coating of gold on the surface of the substrate. In a preferred embodiment, the metal film is from about 50 nm to about 500 nm in thickness. In another embodiment, the metal film is from about 1 nm to about 1  $\mu$ m in thickness.

In alternative embodiments, the coating comprises a composition selected from the group consisting of silicon, silicon oxide, titania, tantalum oxide, silicon nitride, silicon hydride, indium tin oxide, magnesium oxide, alumina, glass, hydroxylated surfaces, and polymers.

If the reactive site comprises a coating between the substrate and the organic thinfilm, then it is understood that the coating must be composed of a material for which a suitable functional group on the organic thinfilm is available. If no such coating is present, then it is understood that the substrate must be composed of a material for which a suitable functional group on the organic thinfilm is available.

It is contemplated that many coatings will require the addition of at least one adhesion layer or mediator between the coating and the substrate. For instance, a layer of titanium or chromium may be desirable between a silicon wafer and a gold coating. In an alternative embodiment, an epoxy glue such as Epo-tek 377®, Epo-tek 301-2®, (Epoxy Technology Inc., Billerica, Massachusetts) may be preferred to aid adherence of the coating to the substrate. Determinations as to what material should be used for the adhesion layer would be obvious to one skilled in the art once materials are chosen for both the substrate and coating. In other embodiments, additional adhesion mediators or interlayers may be necessary to improve the optical properties of the device, for instance, in waveguides for detection purposes.

Deposition or formation of the coating on the substrate (if such coatings are desired) must occur prior to the formation of organic thinfilms thereon.

The organic thinfilm on the reactive sites of the device forms a layer either on the substrate itself or on a coating covering the substrate. The organic thinfilm on which the biological moieties are immobilized is preferably less than about 20 nm thick. In some embodiments of the invention, the organic thinfilm of each of the sites may be less than about 10 nm thick.

A variety of different organic thinfilms are suitable for use in the present invention. Methods for the formation of organic thinfilms include *in situ* growth from the surface, deposition by physisorption, spin-coating, chemisorption, self-assembly, or plasma-initiated polymerization from gas phase. For instance, a hydrogel composed of a material such as dextran can serve as a suitable organic thinfilm on the sites of the device. In one preferred embodiment of the invention,



the organic thinfilm is a lipid bilayer or lipid monolayer. In another preferred embodiment, the organic thinfilm of each of the sites of the device is a monolayer. A monolayer of polyarginine or polylysine adsorbed on a negatively charged substrate or coating is one option for the organic thinfilm. Another option is a disordered monolayer of tethered polymer chains. In a particularly preferred embodiment, the organic thinfilm is a self-assembled monolayer. A monolayer of polylysine is one option for the organic thinfilm. The organic thinfilm is most preferably a self-assembled monolayer which comprises molecules of the formula X-R-Y, wherein R is a spacer, X is a functional group that binds R to the surface, and Y is a functional group for binding proteins onto the monolayer. In an alternative preferred embodiment, the self-assembled monolayer is comprised of molecules of the formula  $(X)_aR(Y)_b$  where a and b are, independently, integers equal to at least one and X, R, and Y are as previously defined. In an alternative preferred embodiment, the organic thinfilm comprises a combination of organic thinfilms such as a combination of a lipid bilayer immobilized on top of a self-assembled monolayer of molecules of the formula X-R-Y. As another example, a monolayer of polylysine can also optionally be combined with a self-assembled monolayer of molecules of the formula X-R-Y (see US Patent No. 5,629,213).

A variety of chemical moieties may function as monolayer molecules of the formula X-R-Y in the device of the present invention. However, three major classes of monolayer formation are preferably used to expose high densities of reactive omega-functionalities on the reactive sites of the device: (i) alkylsiloxane monolayers ("silanes") on hydroxylated and non-hydroxylated surfaces (as taught in, for example, US Patent No. 5,405,766, PCT Publication WO 96/38726, US Patent No. 5,412,087, and US Patent No. 5,688,642); (ii) alkyl-thiol/dialkyldisulfide monolayers on noble metals (preferably Au(111)) (as, for example, described in Allara *et al.*, US 4,690,715; Bamdad *et al.*, US 5,620,850; Wagner *et al.*, *Biophysical Journal*, 1996, 70:2052-2066); and (iii) alkyl monolayer formation on oxide-free passivated silicon (as taught in, for example, Linford *et al.*, *J. Am. Chem. Soc.*, 1995, 117:3145-3155, Wagner *et al.*, *Journal of*

*Structural Biology*, 1997, 119:189-201, US Patent No. 5,429,708). One of ordinary skill in the art, however, will recognize that many possible moieties may be substituted for X, R, and/or Y, dependent primarily upon the choice of substrate, coating, and affinity tag. Many examples of monolayers are described in Ulman, *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self Assembly*, Academic press (1991).

In one embodiment, the monolayer comprises molecules of the formula  $(X)_a R(Y)_b$  wherein a and b are, independently, equal to an integer between 1 and about 200. In a preferred embodiment, a and b are, independently, equal to an integer between 1 and about 80. In a more preferred embodiment, a and b are, independently, equal to 1 or 2. In a most preferred embodiment, a and b are both equal to 1 (molecules of the formula X-R-Y).

If the sites of the invention device comprise a self-assembled monolayer of molecules of the formula  $(X)_a R(Y)_b$ , then R may optionally comprise a linear or branched hydrocarbon chain from about 1 to about 400 carbons long. The hydrocarbon chain may comprise an alkyl, aryl, alkenyl, alkynyl, cycloalkyl, alkaryl, aralkyl group, or any combination thereof. If a and b are both equal to one, then R is typically an alkyl chain from about 3 to about 30 carbons long. In a preferred embodiment, if a and b are both equal to one, then R is an alkyl chain from about 8 to about 22 carbons long and is, optionally, a straight alkane. However, it is also contemplated that in an alternative embodiment, R may readily comprise a linear or branched hydrocarbon chain from about 2 to about 400 carbons long and be interrupted by at least one hetero atom. The interrupting hetero groups can include -O-, -CONH-, -CONHCO-, -NH-, -CSNH-, -CO-, -CS-, -S-, -SO-,  $-(OCH_2CH_2)_n-$  (where  $n=1-20$ ),  $-(CF_2)_n-$  (where  $n=1-22$ ), and the like. Alternatively, one or more of the hydrogen moieties of R can be substituted with deuterium. In alternative, less preferred, embodiments, R may be more than about 400 carbons long.

X may be chosen as any group which affords chemisorption or physisorption of the monolayer onto the surface of the substrate (or the coating, if

present). For instance, if the substrate or coating is a metal or metal alloy, X, at least prior to incorporation into the monolayer, is preferably an asymmetrical or symmetrical disulfide, sulfide, diselenide, selenide, thiol, isonitrile, selenol, a trivalent phosphorus compound, isothiocyanate, isocyanate, xanthanate, thiocarbamate, a phosphine, a amine, thio acid or dithio acid. This embodiment is especially preferred when the substrate, or coating if used, is a noble metal such as gold, silver, or platinum.

If the substrate of the device is a material such as silicon, silicon oxide, indium tin oxide, magnesium oxide, alumina, quartz, glass, or silica, then the device of one embodiment of the invention comprises an X that, prior to incorporation into said monolayer, is a monohalosilane, dihalosilane, trihalosilane, trialkoxysilane, dialkoxysilane, or a monoalkoxysilane. Among these silanes, trichlorosilane and trialkoxysilane are particularly preferred.

In a preferred embodiment of the invention, the substrate is selected from the group consisting of silicon, silicon dioxide, indium tin oxide, alumina, glass, and titania; and X, prior to incorporation into said monolayer, is selected from the group consisting of a monohalosilane, dihalosilane, trihalosilane, trichlorosilane, trialkoxysilane, dialkoxysilane, monoalkoxysilane, carboxylic acid, and phosphate.

In another preferred embodiment of the invention, the substrate of the device is silicon and X is an olefin.

In still another preferred embodiment of the invention, the coating (or the substrate if no coating is present) is titania or tantalum oxide and X is a phosphate.

In other embodiments, the surface of the substrate (or coating thereon) is composed of a material such as titanium oxide, tantalum oxide, indium tin oxide, magnesium oxide, or alumina where X is a carboxylic acid or alkylphosphoric acid. Alternatively, if the surface of the substrate (or coating thereon) of the device is copper, then X may optionally be a hydroxamic acid.

If the substrate used in the invention is a polymer, then in many cases a coating on the substrate such as a copper coating will be included in the device.

An appropriate functional group X for the coating would then be chosen for use in the device. In an alternative embodiment comprising a polymer substrate, the surface of the polymer may be plasma-modified to expose desirable surface functionalities for monolayer formation. For instance, EP 780423 describes the use of a monolayer molecule that has an alkene X functionality on a plasma exposed surface. Still another possibility for the invention device comprised of a polymer is that the surface of the polymer on which the monolayer is formed is functionalized due to copolymerization of appropriately functionalized precursor molecules.

Another possibility is that prior to incorporation into the monolayer, X can be a free-radical-producing moiety. This functional group is especially appropriate when the surface on which the monolayer is formed is a hydrogenated silicon surface. Possible free-radical producing moieties include, but are not limited to, diacylperoxides, peroxides, and azo compounds. Alternatively, unsaturated moieties such as unsubstituted alkenes, alkynes, cyano compounds and isonitrile compounds can be used for X, if the reaction with X is accompanied by ultraviolet, infrared, visible, or microwave radiation.

In alternative embodiments, X, prior to incorporation into the monolayer, may be a hydroxyl, carboxyl, vinyl, sulfonyl, phosphoryl, silicon hydride, or an amino group.

The component, Y, of the monolayer is responsible for binding a biological moiety onto the monolayer. In a preferred embodiment of the invention, the Y group is either highly reactive (activated) towards the biological moiety (or its affinity tag) or is easily converted into such an activated form. In a preferred embodiment, the coupling of Y with the biological moiety occurs readily under normal physiological conditions not detrimental to the biological activity of the biological moiety. The functional group Y may either form a covalent linkage or a noncovalent linkage with the biological moiety (or its affinity tag, if present). In a preferred embodiment, the functional group Y forms a covalent linkage with the biological moiety or its affinity tag. It is understood that following the attachment

of the biological moiety (with or without an affinity tag) to Y, the chemical nature of Y may have changed. Upon attachment of the biological moiety, Y may even have been removed from the organic thinfilm.

In one embodiment of the present invention, Y is a functional group that is activated *in situ* before attachment of the biological moiety. Possibilities for this type of functional group include, but are not limited to, such simple moieties such as a hydroxyl, carboxyl, amino, aldehyde, carbonyl, methyl, methylene, alkene, alkyne, carbonate, aryl iodide, or a vinyl group. Appropriate modes of activation for these simple functional groups would be known by one of ordinary skill in the art. Alternatively, Y can comprise a functional group that requires photoactivation prior to becoming activated enough to trap the biological moiety.

In an especially preferred embodiment of the device of the present invention, Y is a highly reactive functional moiety compatible with monolayer formation and needs no *in situ* activation prior to reaction with the biological moiety or its affinity tag. Such possibilities for Y include, but are not limited to, maleimide, N-hydroxysuccinimide (Wagner et al., *Biophysical Journal*, 1996, 70:2052-2066), nitrilotriacetic acid (US Patent No. 5,620,850), activated hydroxyl, haloacetyl, bromoacetyl, iodoacetyl, activated carboxyl, hydrazide, epoxy, aziridine, sulfonylchloride, trifluoromethyldiaziridine, pyridyldisulfide, N-acyl-imidazole, imidazolecarbamate, vinylsulfone, succinimidylcarbonate, arylazide, anhydride, diazoacetate, benzophenone, isothiocyanate, isocyanate, imidoester, fluorobenzene, and biotin.

Figure 4 shows one example of a monolayer on a substrate 3. In this example, substrate 3 comprises glass. The monolayer is thioreactive because it bears a maleimidyl functional group Y.

Figure 5 shows another example of a monolayer on a substrate 3 which is silicon. In this case, however, a thinfilm gold coating 5 covers the surface of the substrate 3. Also, in this embodiment, a titanium adhesion interlayer 6 is used to adhere the coating 5 to the substrate 3. This monolayer is aminoreactive because it bears an N-hydroxysuccinimidyl functional group Y.

In an alternative embodiment, Y is selected from the group of simple functional moieties. Possible Y functional groups include, but are not limited to, -OH, -NH<sub>2</sub>, -COOH, -COOR, -RSR, -PO<sub>4</sub><sup>-3</sup>, -OSO<sub>3</sub><sup>-2</sup>, -SO<sub>3</sub>-, -COO-, -SOO-, -CONR<sub>2</sub>, -CN, -NR<sub>2</sub>, and the like.

The monolayer molecules of the present invention can optionally be assembled on the surface in parts. In other words, the monolayer need not necessarily be constructed by chemisorption or physisorption of molecules of the formula X-R-Y to the surface of the substrate (or coating). Instead, in one embodiment, X may be chemisorbed or physisorbed to the surface of the substrate (or coating) alone first. Then, R or even just individual components of R can be attached to X through a suitable chemical reaction. Upon completion of addition of the spacer R to the X moiety already immobilized on the surface, Y can be attached to the ends of the monolayer molecule through a suitable covalent linkage.

Not all monolayer molecules at a given reactive site need to be identical. Some may consist of mixed monolayers. For instance, the monolayer of an individual reactive site may optionally comprise at least two different X-R-Y molecules. This second X-R-Y molecule may immobilize the same or a different biological moiety. In addition, many of the monolayer molecules, X-R-Y, of a reactive site may have failed to attach any biological moiety.

As another alternative of the invention, the monolayer of an individual reactive site can comprise a second organic molecule that is of the formula, X-R-V where R is the spacer, X is the functional group that binds R to the surface, and V is a moiety resistant to the non-specific binding of biomolecules. One of ordinary skill in the art will recognize that the possibilities for V will vary depending upon the nature of the biological moiety chosen for the sites of the device. For instance, functional groups V which are resistant to non-specific protein binding are used if the immobilized biological moiety of the device comprises protein. The nature of V will be somewhat dependent upon the type of

proteins and solutions used. For instance, V may comprise a hydroxyl, saccharide, or oligo/polyethylene glycol moiety (EP Publication 780423).

As a still further alternative of the invention device, the device may further comprise at least one unreactive site devoid of any biological moiety that comprises a monolayer of molecules of the formula X-R-V, where R is the spacer, X is the functional group that binds R to the surface, and V is the moiety resistant to the non-specific binding of biomolecules. In this embodiment, the unreactive site does not comprise any monolayers of molecules of the formula X-R-Y.

Regardless of the nature of the monolayer molecules, in some cases it can be desirable to provide crosslinking between molecules of the monolayer. In general, this confers additional stability to the monolayer. Methods of crosslinking such monolayers are known to those skilled in the art (see Ullman, *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self-Assembly*, Academic Press (1991)).

In addition to facilitating binding of the biological moiety to the substrate, functionalization of the substrate with organic thinfilms is desirable for other reasons as well. Many biological moieties and protein, in particular, are susceptible to disruption of their bioactivities at surface interfaces. Proteins are prone to both denaturation and undesirable, non-specific binding at the solid/liquid interface. Other biological moieties such as small molecule ligands may have less problematic interactions with the substrate surface interface, but upon approach of the biological binding partner, presumably a protein, to the small molecule in an assay, problems of inactivation become highly relevant. A highly-ordered organic monolayer can effectively "carpet" the surface of the substrate or coating, protecting the biological moiety from contact with the surface. These highly-ordered, self-assembled monolayers are preferred in the present invention. Additionally, the spacer R creates distance between the immobilized biological moiety and the surface.

Following formation of organic thinfilm on the reactive sites of the invention device, the biological moieties are immobilized on the monolayers. A

solution containing the biological moiety to be immobilized can be exposed to the bioreactive, organic thinfilm covered sites of the microdevice by either dispensing the solution by means of microfabricated adapter systems with integrated microcapillaries and entry/exit ports. Such a dispensing mechanism would be suitable, for instance, if the reactive sites of the device were in covered, parallel microchannels. Alternatively, the biological moieties may be transferred to uncovered sites of the device by using one of the arrayers based on capillary dispensing systems which are well known in the art and even commercially available. These dispensing systems are preferably automated and computer-aided. A description of and building instructions for an example of a microarrayer comprising an automated capillary system can be found on the internet at <http://cmgm.stanford.edu/pbrown/array.html> and <http://cmgm.stanford.edu/pbrown/mguide/index.html>. The use of other printing techniques is also anticipated. Following attachment of the biological moieties to the monolayer, unreacted Y-functional groups are preferably quenched prior to use of the device.

In an alternative embodiment of the invention, the reactive sites of the device are not contained within microchannels. For instance, the reactive sites of the invention device may instead form an array of reactive sites like some of those described in the co-pending U.S. patent applications "Arrays of Protein-Capture Agents and Methods of Use Thereof", filed on July 14, 1999, with the identifier 24406-0006, for the inventors Peter Wagner, Steffen Nock, Dana Ault-Riche, and Christian Itin, and "Arrays of Proteins and Methods of Use Thereof", filed on July 14, 1999, with the identifier 24406-0004 P1, for the inventors Peter Wagner, Dana Ault-Riche, Steffen Nock, and Christian Itin, both of which are herein incorporated by reference in their entirety.

(c) Affinity tags and immobilization of the biological moieties.

In a preferred embodiment, the reactive sites of the device further comprise an affinity tag that enhances immobilization of the biological moiety onto the



organic thinfilm. The use of an affinity tag to immobilize the biological moiety typically provides several advantages. An affinity tag can confer enhanced binding or reaction of the biological moiety with the functionalities on the organic thinfilm, such as Y if the organic thinfilm is an X-R-Y monolayer as previously described. This enhancement effect may be either kinetic or thermodynamic. The affinity tag/thinfilm combination used on the reactive sites of the device preferably allows for immobilization of the biological moieties in a manner which does not require harsh reaction conditions that are adverse to the stability or function of the biological moiety. In most embodiments, immobilization to the organic thinfilm in aqueous, biological buffers is ideal.

An affinity tag also preferably offers immobilization on the organic thinfilm that is specific to a designated site or location on the biological moiety (site-specific immobilization). For this to occur, attachment of the affinity tag to the biological moiety must be site-specific. Site-specific immobilization helps ensure that the active site or binding site of the immobilized biological moiety, such as the antigen-binding site of an antibody, remains accessible to ligands in solution. Another advantage of immobilization through affinity tags is that it allows for a common immobilization strategy to be used with multiple, different biological moieties.

The affinity tag is optionally attached directly, either covalently or noncovalently, to the biological moiety. In an alternative embodiment, however, the affinity tag is either covalently or noncovalently attached to an adaptor which is either covalently or noncovalently attached to the biological moiety.

In a preferred embodiment, the affinity tag comprises at least one amino acid. The affinity tag may be a polypeptide comprising at least two amino acids which is reactive with the functionalities of the organic thinfilm. Alternatively, the affinity tag may be a single amino acid which is reactive with the organic thinfilm. Examples of possible amino acids which could be reactive with an organic thinfilm include cysteine, lysine, histidine, arginine, tyrosine, aspartic acid, glutamic acid, tryptophan, serine, threonine, and glutamine. If the biological

moiety of a reactive site to be immobilized is a protein, then the polypeptide or amino acid affinity tag is preferably expressed as a fusion protein with the biological moiety. Amino acid affinity tags provide either a single amino acid or a series of amino acids that can interact with the functionality of the organic thinfilm, such as the Y-functional group of the self-assembled monolayer molecules. Amino acid affinity tags can be readily introduced into recombinant proteins to facilitate oriented immobilization by covalent binding to the Y-functional group of a monolayer or to a functional group on an alternative organic thinfilm.

The affinity tag may optionally comprise a poly(amino acid) tag. A poly(amino acid) tag is a polypeptide that comprises from about 2 to about 100 residues of a single amino acid, optionally interrupted by residues of other amino acids. For instance, the affinity tag may comprise a poly-cysteine, polylysine, poly-arginine, or poly-histidine. Amino acid tags are preferably composed of two to twenty residues of a single amino acid, such as, for example, histidines, lysines, arginines, cysteines, glutamines, tyrosines, or any combination of these. According to a preferred embodiment, an amino acid tag of one to twenty amino acids includes at least one to ten cysteines for thioether linkage; or one to ten lysines for amide linkage; or one to ten arginines for coupling to vicinal dicarbonyl groups. One of ordinary skill in the art can readily pair suitable affinity tags with a given functionality on an organic thinfilm.

The position of the amino acid tag can be at the amino-terminus or the carboxy-terminus of the biological moiety of a reactive site which is a protein, or anywhere in-between, as long as the active site or binding site of the biological moiety remains in a position accessible for ligand interaction. Where compatible with the protein chosen, affinity tags introduced for protein purification are preferentially located at the C-terminus of the recombinant protein to ensure that only full-length proteins are isolated during protein purification. For instance, if intact antibodies are used on the reactive sites, then the attachment point of the affinity tag on the antibody is preferably located at a C-terminus of the effector

(Fc) region of the antibody. If scFvs are used on the reactive sites, then the attachment point of the affinity tag is also preferably located at the C-terminus of the molecules.

Affinity tags may also contain one or more unnatural amino acids. Unnatural amino acids can be introduced using suppressor tRNAs that recognize stop codons (*i.e.*, amber) (Noren *et al.*, *Science*, 1989, 244:182-188; Ellman *et al.*, *Methods Enzym.*, 1991, 202:301-336; Cload *et al.*, *Chem. Biol.*, 1996, 3:1033-1038). The tRNAs are chemically amino-acylated to contain chemically altered ("unnatural") amino acids for use with specific coupling chemistries (*i.e.*, ketone modifications, photoreactive groups).

In an alternative embodiment the affinity tag can comprise an intact protein, such as, but not limited to, glutathione S-transferase, an antibody, avidin, or streptavidin.

Other protein conjugation and immobilization techniques known in the art may be adapted for the purpose of attaching affinity tags to the biological moiety. For instance, in an alternative embodiment of the device, the affinity tag may be an organic bioconjugate which is chemically coupled to the biological moiety of interest. Biotin or antigens may be chemically cross linked to the biological moiety. Alternatively, a chemical crosslinker may be used that attaches a simple functional moiety such as a thiol or an amine to the surface of a biological moiety to be immobilized on a reactive site of the device. Alternatively, protein synthesis or protein ligation techniques known to those skilled in the art may be used to attach an affinity tag to a biological moiety which is a protein. For instance, intein-mediated protein ligation may optionally be used to attach the affinity tag to the biological moiety (Mathys, *et al.*, *Gene* 231:1-13, 1999; Evans, *et al.*, *Protein Science* 7:2256-2264, 1998).

In an alternative embodiment of the invention, the organic thinfilm of each of the reactive sites comprises, at least in part, a lipid monolayer or bilayer, and the affinity tag comprises a membrane anchor. Optionally, the lipid monolayer or bilayer is immobilized on a self-assembled monolayer.

Figure 6 shows a biological moiety 10 immobilized on a monolayer 7 on a substrate 3. An affinity tag 8 connects the biological moiety 10 to the monolayer 7. The monolayer 7 is formed on a coating 5 separated from the surface of the substrate 3 by an interlayer 6.

Figure 7 shows a cross section of a biomolecule-coated microchannel of one embodiment of a microchannel array device. The microchannel 1 is covered by a glass cover 2. The walls of the microchannel are comprised of substrate 3, coated first with an interlayer 6, then with a coating 5, then with an organic monolayer 7 and finally, with the biological moiety 10 via the affinity tag 8.

In an alternative embodiment of the invention, no affinity tag is used to immobilize the biological moieties onto the organic thinfilm. An amino acid, nucleotide, or other moiety (such as a carbohydrate moiety) inherent to the biological moiety itself may instead be used to tether the protein to the reactive group of the organic thinfilm. In preferred embodiments, the immobilization is site-specific with respect to the location of the site of immobilization on the biological moiety. For instance, the sulfhydryl group on the C-terminal region of the heavy chain portion of a Fab' fragment generated by pepsin digestion of an antibody, followed by selective reduction of the disulfide between monovalent Fab' fragments, may be used as the affinity tag. Alternatively, a carbohydrate moiety on the Fc portion of an intact antibody can be oxidized under mild conditions to an aldehyde group suitable for immobilizing the antibody on a monolayer via reaction with a hydrazide-activated Y group on the monolayer. Examples of immobilization of proteins without any affinity tag can be found in Wagner *et al.*, *Biophys. J.*, 70:2052-2066, 1996.

(d) Adaptors.

Another embodiment of the devices of the present invention comprises an adaptor that links the affinity tag to the immobilized biological moiety. The additional spacing of the protein from the surface of the substrate (or coating) that is afforded by the use of an adaptor is particularly advantageous since some

biological moieties such as proteins are known to be prone to surface inactivation. The adaptor may optionally afford some additional advantages as well. For instance, the adaptor may help facilitate the attachment of the biological moiety to the affinity tag. In another embodiment, the adaptor may help facilitate the use of a particular detection technique with the device. One of ordinary skill in the art will be able to choose an adaptor which is appropriate for a given affinity tag. For instance, if the affinity tag is streptavidin, then the adaptor could be a biotin molecule that is chemically conjugated to the protein which is to be immobilized.

In a preferred embodiment, the adaptor is a protein. In a preferred embodiment, the affinity tag, adaptor, and biological moiety together compose a fusion protein. Such a fusion protein may be readily expressed using standard recombinant DNA technology. Adaptors which are proteins are especially useful to increase the solubility of the protein of interest and to increase the distance between the surface of the substrate or coating and the protein of interest. Use of an adaptor which is a protein can also be very useful in facilitating the preparative steps of protein purification by affinity binding prior to immobilization on the device. Examples of possible adaptors which are proteins include glutathione-S-transferase (GST), maltose-binding protein, chitin-binding protein, thioredoxin, green-fluorescent protein (GFP). GFP can also be used for quantification of surface binding. If the biological moiety immobilized on the reactive sites of the device is an antibody or antibody fragment comprising an Fc region, then the adaptor may optionally be protein G, protein A, or recombinant protein A/G (a gene fusion product secreted from a non-pathogenic form of *Bacillus* which contains four Fc binding domains from protein A and two from protein G).

Figure 8 shows a biological moiety 10 immobilized on a monolayer 7 via both an affinity tag 8 and an adaptor molecule 9. The monolayer 7 has been formed on a coating 5 on a substrate 3. An interlayer 6 is also used between the coating 5 and the substrate 3.

(e) The immobilized biological moieties and preparation thereof.

In one embodiment of the present invention, the biological moiety of one reactive site differs from the biological moiety of a second reactive site on the same device. In a preferred embodiment, the device comprises at least about 10 different immobilized biological moieties. In an especially preferred embodiment, the device comprises at least about 100 different immobilized biological moieties.

In a preferred embodiment, the biological moieties immobilized on the sites of the invention device are proteins. Although proteins are the preferred biological moieties of the present invention, the immobilized biological moiety may optionally instead comprise a polynucleotide, a peptide nucleic acid, a hormone, an antigen, an epitope, or any small organic molecule which either has or is suspected of having a physiological function.

In another embodiment of the present invention, although the biological moiety of one reactive site is different from that of another, the two biological moieties are related. In a preferred embodiment the biological moieties are members of the same protein family. The different biological moieties may be functionally related or just suspected of being functionally related. In another embodiment, however, the function of the biological moieties may not be entirely known. In these cases, the different biological moieties may either share a similarity in structure or sequence or be suspected of sharing a similarity in structure or sequence. In one embodiment, the different immobilized biological moieties may simply be fragments of different members of the same protein family. In another embodiment, the biological moieties may be known isozymes.

Examples of protein families include, but are not limited to, a receptor families (examples: growth factor receptors, catecholamine receptors, amino acid derivative receptors, cytokine receptors, lectins), ligand families (examples: cytokines, serpins), enzyme families (examples: proteases, kinases, phosphatases, ras-like GTPases, hydrolases), and transcription factors (examples: steroid hormone receptors, heat-shock transcription factors, zinc-finger proteins, leucine-zipper proteins, homeodomain proteins). In one embodiment, the different immobilized proteins are all HIV proteases or hepatitis C virus (HCV) proteases.

In other embodiments of the invention, the immobilized proteins on the reactive sites of the invention device are all hormone receptors, neurotransmitter receptors, extracellular matrix receptors, antibodies, DNA-binding proteins, intracellular signal transduction modulators and effectors, apoptosis-related factors, DNA synthesis factors, DNA repair factors, DNA recombination factors, or cell-surface antigens.

In an alternative preferred embodiment, the biological moieties of the sites of the invention device are protein-capture agents. In another preferred embodiment, the biological moieties of the reactive sites or microchannels of the device are all antibodies or antibody fragments.

In an alternative embodiment of the invention device, the biological moieties of the different reactive sites on the device are identical to one another.

The biological moieties immobilized on the device may be produced by any of the variety of means known to those of ordinary skill in the art.

In preparation for immobilization to the sites of the devices of the present invention, a biological moiety which is a protein can optionally be expressed from recombinant DNA either *in vivo* or *in vitro*. The cDNA of the protein to be immobilized on the device is cloned into an expression vector (many examples of which are commercially available) and introduced into cells of the appropriate organism for expression. A broad range of host cells and expression systems may be used to produce the proteins to be immobilized on the device. For *in vivo* expression of the proteins, cDNAs can be cloned into commercial expression vectors (Qiagen, Novagen, Clontech, for example) and introduced into an appropriate organism for expression. Expression *in vivo* may be done in bacteria (for example, *Escherichia coli*), plants (for example, *Nicotiana tabacum*), lower eukaryotes (for example, *Saccharomyces cerevisiae*, *Saccharomyces pombe*, *Pichia pastoris*), or higher eukaryotes (for example, baculovirus-infected insect cells, insect cells, mammalian cells). For *in vitro* expression PCR-amplified DNA sequences are directly used in coupled *in vitro* transcription/translation systems (for instance: *Escherichia coli* S30 lysates from T7 RNA polymerase expressing,

preferably protease-deficient strains; wheat germ lysates; reticulocyte lysates (Promega, Pharmacia, Panvera)). The choice of organism for optimal expression depends on the extent of post-translational modifications (*i.e.*, glycosylation, lipid-modifications) desired. One of ordinary skill in the art will be able to readily choose which host cell type is most suitable for the protein to be immobilized and application desired.

DNA sequences encoding amino acid affinity tags and adaptor protein sequences are engineered into the expression vectors such that the genes of interest can be cloned in frame either 5' or 3' of the DNA sequence encoding the affinity tag and adaptor.

The expressed proteins are purified by affinity chromatography using commercially available resins.

Preferably, production of families of related proteins involves parallel processing from cloning to protein expression and protein purification. cDNAs for the protein of interest will be amplified by PCR using cDNA libraries or EST (expressed sequence tag) clones as templates. Any of the *in vitro* or *in vivo* expression systems described above can then be used for expression of the proteins to be immobilized on the device.

*Escherichia coli*-based protein expression is generally the method of choice for soluble proteins that do not require extensive post-translational modifications for activity. Extracellular or intracellular domains of membrane proteins will be fused to protein adaptors for expression and purification.

The entire approach can be performed using 96-well assay plates. PCR reactions are carried out under standard conditions. Oligonucleotide primers contain unique restriction sites for facile cloning into the expression vectors. Alternatively, the TA cloning system (Clontech) can be used. Expression vectors contain the sequences for affinity tags and the protein adaptors. PCR products are ligated into the expression vectors (under inducible promoters) and introduced into the appropriate competent *Escherichia coli* strain by calcium-dependent transformation (strains include: XL-1 blue, BL21, SG13009(lon-)). Transformed



*Escherichia coli* cells are plated and individual colonies transferred into 96-array blocks. Cultures are grown to mid-log phase, induced for expression, and cells collected by centrifugation. Cells are resuspended containing lysozyme and the membranes broken by rapid freeze/thaw cycles, or by sonication. Cell debris is removed by centrifugation and the supernatants transferred to 96-tube arrays. The appropriate affinity matrix is added, protein of interest bound and nonspecifically bound proteins removed by repeated washing steps using 12 - 96 pin suction devices and centrifugation. Alternatively, magnetic affinity beads and filtration devices can be used (Qiagen). The proteins are eluted and transferred to a new 96-well array. Protein concentrations are determined and an aliquot of each protein is spotted onto a nitrocellulose filter and verified by Western analysis using an antibody directed against the affinity tag. The purity of each sample is assessed by SDS-PAGE and silver staining or mass spectrometry. Proteins are snap-frozen and stored at  $-80^{\circ}\text{C}$ .

*Saccharomyces cerevisiae* allows for core glycosylation and lipid modifications of proteins. The approach described above for *Escherichia coli* can be used with slight modifications for transformation and cell lysis. Transformation of *Saccharomyces cerevisiae* is by lithium-acetate and cell lysis is either by lyticase digestion of the cell walls followed by freeze-thaw, sonication or glass-bead extraction. Variations of post-translational modifications can be obtained by different yeast strains (i.e. *Saccharomyces pombe*, *Pichia pastoris*).

The advantage of the baculovirus system or mammalian cells are the wealth of post-translational modifications that can be obtained. The baculovirus system requires cloning of viruses, obtaining high titer stocks and infection of liquid insect cell suspensions (cells are SF9, SF21). Mammalian cell-based expression requires transfection and cloning of cell lines. Soluble proteins are collected from the medium while intracellular or membrane bound proteins require cell lysis (either detergent solubilization, freeze-thaw). Proteins can then be purified analogous to the procedure described for *Escherichia coli*.

For *in vitro* translation the system of choice is *Escherichia coli* lysates obtained from protease-deficient and T7 RNA polymerase overexpressing strains. *Escherichia coli* lysates provide efficient protein expression (30-50 µg/ml lysate). The entire process is carried out in 96-well arrays. Genes of interest are amplified by PCR using oligonucleotides that contain the gene-specific sequences containing a T7 RNA polymerase promoter and binding site and a sequence encoding the affinity tag. Alternatively, an adaptor protein can be fused to the gene of interest by PCR. Amplified DNAs can be directly transcribed and translated in the *Escherichia coli* lysates without prior cloning for fast analysis. The proteins are then isolated by binding to an affinity matrix and processed as described above.

Alternative systems which may be used include wheat germ extracts and reticulocyte extracts. *In vitro* synthesis of membrane proteins and or post-translationally modified proteins will require reticulocyte lysates in combination with microsomes.

In one preferred embodiment of the invention, the proteins immobilized on the sites of the device are antibodies. Optionally, the immobilized proteins may be monoclonal antibodies. The production of monoclonal antibodies against specific protein targets is routine using standard hybridoma technology. In fact, numerous monoclonal antibodies are available commercially.

As an alternative to obtaining antibodies or antibody fragments which have been produced by cell fusion or from continuous cell lines, the antibody moieties may be expressed in bacteriophage. Such antibody phage display technologies are well known to those skilled in the art. The bacteriophage expression systems allow for the random recombination of heavy- and light-chain sequences, thereby creating a library of antibody sequences which can be selected against the desired antigen. The expression system can be based on bacteriophage  $\lambda$  or, more preferably, on filamentous phage. The bacteriophage expression system can be used to express Fab fragments, Fv's with an engineered intermolecular disulfide bond to stabilize the  $V_H$ - $V_L$  pair (dsFv's), scFv's, or diabody fragments.

The antibody genes of the phage display libraries may be from pre-immunized donors. For instance, the phage display library could be a display library prepared from the spleens of mice previously immunized with a mixture of proteins (such as a lysate of human T-cells). Immunization can optionally be used to bias the library to contain a greater number of recombinant antibodies reactive towards a specific set of proteins (such as proteins found in human T-cells). Alternatively, the library antibodies may be derived from naive or synthetic libraries. The naive libraries have been constructed from spleens of mice which have not been contacted by external antigen. In a synthetic library, portions of the antibody sequence, typically those regions corresponding to the complementarity determining regions (CDR) loops, have been mutagenized or randomized.

The phage display method involves batch-cloning the antibody gene library into a phage genome as a fusion to the gene encoding one of the phage coat proteins (pIII, pVI, or pVIII). The pIII phage protein gene is preferred. When the fusion product is expressed it is incorporated into the mature phage coat. As a result, the antibody is displayed as a fusion on the surface of the phage and is available for binding and hence, selection, on a target protein. Once a phage particle is selected as bearing an antibody-coat protein fusion with the desired affinity towards the target protein, the genetic material within the phage particle which corresponds to the displayed antibody can be amplified and sequenced or otherwise analyzed.

In a preferred embodiment, a phagemid is used as the expression vector in the phage display procedures. A phagemid is a small plasmid vector that carries gene III with appropriate cloning sites and a phage packaging signal and contains both host and phage origins of replication. The phagemid is unable to produce a complete phage as the gene III fusion is the only phage gene encoded on the phagemid. A viable phage can be produced by infecting cells containing the phagemid with a helper phage containing a defective replication origin. A hybrid phage emerges which contains all of the helper phage proteins as well as the gene III-rAb fusion. The emergent phage contains the phagemid DNA only.

In a preferred embodiment of the invention, the recombinant antibodies used in phage display methods of preparing antibody fragments for the devices of the invention are expressed as genetic fusions to the bacteriophage gene III protein on a phagemid vector. For instance, the antibody variable regions encoding a single-chain Fv fragment can be fused to the amino terminus of the gene III protein on a phagemid. Alternatively, the antibody fragment sequence could be fused to the amino terminus of a truncated pIII sequence lacking the first two N-terminal domains. The phagemid DNA encoding the antibody-pIII fusion is preferably packaged into phage particles using a helper phage such as M13KO7 or VCS-M13, which supplies all structural phage proteins.

To display Fab fragments on phage, either the light or heavy (Fd) chain is fused via its C-terminus to pIII. The partner chain is expressed without any fusion to pIII so that both chains can associate to form an intact Fab fragment.

Any method of selection may be used which separates those phage particles which do bind the target protein from those which do not. The selection method must also allow for the recovery of the selected phages. Most typically, the phage particles are selected on an immobilized target protein. Some phage selection strategies known to those skilled in the art include the following: panning on an immobilized antigen; panning on an immobilized antigen using specific elution; using biotinylated antigen and then selecting on a streptavidin resin or streptavidin-coated magnetic beads; affinity purification; selection on Western blots (especially useful for unknown antigens or antigens difficult to purify); *in vivo* selection; and pathfinder selection. If the selected phage particles are amplified between selection rounds, multiple iterative rounds of selection may optionally be performed.

Elution techniques will vary depending upon the selection process chosen, but typical elution techniques include washing with one of the following solutions: HCl or glycine buffers; basic solutions such as triethylamine; chaotropic agents; solutions of increased ionic strength; or DTT when biotin is linked to the antigen by a disulfide bridge. Other typical methods of elution

include enzymatically cleaving a protease site engineered between the antibody and gene III, or by competing for binding with excess antigen or excess antibodies to the antigen.

In the preparation of the devices of the invention, phage display methods analogous to those used for antibody fragments may be used for other proteins which are to be immobilized on a device of the invention as long as the protein is of suitable size to be incorporated into the phagemid or alternative vector and expressed as a fusion with a bacteriophage coat protein. Phage display techniques using non-antibody libraries typically make use of some type of protein host scaffold structure which supports the variable regions. For instance,  $\beta$ -sheet proteins,  $\alpha$ -helical handle proteins, and other highly constrained protein structures have been used as host scaffolds.

Alternative display vectors may also be used to produce proteins which are immobilized on the sites of the device. Polysomes, stable protein-ribosome-mRNA complexes, can be used to replace live bacteriophage as the display vehicle for recombinant antibody fragments or other proteins (Hanes and Pluckthun, *Proc. Natl. Acad. Sci USA*, 94:4937-4942, 1997). The polysomes are formed by preventing release of newly synthesized and correctly folded protein from the ribosome. Selection of the polysome library is based on binding of the antibody fragments or other proteins which are displayed on the polysomes to the target protein. mRNA which encodes the displayed protein or antibody having the desired affinity for the target is then isolated. Larger libraries may be used with polysome display than with phage display.

(e) Uses of the devices.

Methods for using the devices of the present invention are provided by other aspects of the invention. The devices of the present invention are particularly well-suited for use in drug development, such as in high-throughput drug screening. Other uses include medical diagnostics and biosensors. The devices of the invention are also useful for functional proteomics. In each case, a

plurality of biological moieties or drug candidates or analytes can be screened for potential biological interactions in parallel.

In one aspect of the invention, a method for screening a plurality of different biological moieties in parallel for their ability to interact with a component of a fluid sample is provided. This method comprises delivering the fluid sample to the reactive sites of one of the invention devices where each of the different biological moieties is immobilized on a different site of the device, and then detecting for the interaction of the component with the immobilized biological moiety at each reactive site. In a preferred embodiment, each of the reactive sites is in a microchannel oriented parallel to microchannels of other reactive sites on the device, wherein the microchannels are microfabricated into or onto the substrate.

The invention device is suitable for assaying a catalytic reaction of an enzyme, a binding event, or even a translocation by a membrane protein through a lipid bilayer. Possible interactions towards which the present invention may be directed include, but are not limited to, antibody/antigen, antibody/hapten, enzyme/substrate, carrier protein/substrate, lectin/carbohydrate, receptor/hormone, receptor/effector, protein/DNA, protein/RNA, complementary strands of nucleic acid, repressor/inducer, or the like. The assayed interaction may be between a potential drug candidate and a plurality of potential drug targets. For instance, a synthesized organic compound may be tested for its ability to act as an inhibitor to a family of immobilized receptors. The devices are also highly suitable for assaying for protein-protein interactions in general.

One embodiment of the present invention provides for a method of screening a plurality of biological moieties in parallel for their ability to react with a component of a fluid sample, comprising delivering the fluid sample to the reactive sites of a device of the present invention, wherein each of the different biological moieties is immobilized on a different reactive site of the device and detecting, either directly or indirectly, for formation of product of the reaction of the component with the immobilized biological moiety at each reactive site.

Another embodiment of the invention provides a method for screening a plurality of biological moieties in parallel for their ability to bind a component of a fluid sample. This method comprises the following steps: delivering the fluid sample to the reactive sites of the invention device, wherein each of the different biological moieties is immobilized on a different site of the device; optionally, washing the reactive site remove unbound or nonspecifically bound components of the sample from the reactive sites; and detecting, either directly or indirectly, for the presence or amount of the component retained at each reactive site.

An alternative method for screening a plurality of biological moieties for their ability to bind a component of a fluid sample comprises first adding a known ligand of the biological moieties to the fluid sample and then delivering the fluid sample to the reactive sites of the invention device, where each of the different biological moieties is immobilized on a different site of the device. As an optional next step, the reactive sites may be washed with fluid that does not contain either the known ligand or the component in order to elute unbound or nonspecifically bound molecules of the known ligand and the component (or other components from the sample) from the reactive sites of the device. A final step of the method comprises detecting the presence or amount of the known ligand retained at each reactive site, and comparing retention of the known ligand at each reactive site with retention of the known ligand at the same or an identical reactive site in the absence of the component.

A wide range of detection methods is applicable to the methods of the invention. As desired, detection may be either quantitative or qualitative. The invention device can be interfaced with optical detection methods such as absorption in the visible or infrared range, chemoluminescence, and fluorescence (including lifetime, polarization, fluorescence correlation spectroscopy (FCS), and fluorescence-resonance energy transfer (FRET)). Furthermore, other modes of detection such as those based on optical waveguides (PCT Publication WO 96/26432 and U.S. Patent No. 5,677,196), surface plasmon resonance, surface charge sensors, and surface force sensors are compatible with many embodiments

of the invention. Alternatively, technologies such as those based on Brewster angle microscopy (Schaaf *et al.*, *Langmuir*, 3:1131-1135 (1987)) and ellipsometry (U.S. Patent Nos. 5,141,311 and 5,116,121; Kim, *Macromolecules*, 22:2682-2685 (1984)) can be used in conjunction with the devices of the invention. Quartz crystal microbalances and desorption processes (see for example, U.S. Patent No. 5,719,060) provide still other alternative detection means suitable for at least some embodiments of the invention device. An example of an optical biosensor system compatible both with some devices of the present invention and a variety of non-label detection principles including surface plasmon resonance, total internal reflection fluorescence (TIRF), Brewster Angle microscopy, optical waveguide lightmode spectroscopy (OWLS), surface charge measurements, and ellipsometry can be found in U.S. Patent No. 5,313,264.

Figure 9 shows a schematic diagram of one type of fluorescence detection unit which may be used to monitor the interaction of immobilized biological moieties of a microchannel array with an analyte. In the illustrated detection unit, the microchannel array device 21 is positioned on a base plate 20. Light from a 100W mercury arc lamp 25 is directed through an excitation filter 24 and onto a beam splitter 23. The light is then directed through a lens 22, such as a Micro Nikkor 55 mm 1:2.8 lens, and onto the microchannels of the device 21. Fluorescence emission from the device returns through the lens 22 and the beam splitter 23. After also passing through an emission filter 26, the emission is received by a cooled CCD camera 27, such as the Slowsan TE/CCD-1024SF&SB (Princeton Instruments). The camera is operably connected to a CPU 28, which is, in turn, operably connected to a VCR 29 and monitor 30.

To test the specificity of a drug candidate, its interaction with multiple members of a protein family is determined. Members of the protein family are separately immobilized in microchannels. The drug candidate's ability to interfere with protein activity, such as binding, catalytic conversion, or translocation of a ligand through a lipid bilayer, is then determined.



In another example, to test a drug candidate's ability to interfere with a protein binding event, the drug candidate and a known ligand of a member of the protein family that is labeled by a chemically-conjugated fluorescent moiety are delivered in a fluid sample into each microchannel of the device. After a short incubation period, the microchannels are flushed with fluid which lacks both the drug candidate and the ligand. The amount of fluorescent ligand remaining in each of the microchannels (and presumably bound to the protein molecules of that microchannel) can then be detected by using a fluorescence detector / quantifier with optical access to the reactive site, either through a transparent or translucent cover or substrate.

To test a drug candidate's ability to interfere with a catalytic conversion of a ligand, drug candidate and ligand are delivered into the microchannel in a fluid sample and changes in the chromogenic or fluorescent properties can be detected by using an optical detector / quantifier with optical access to the reactive site, either through a transparent or translucent cover or substrate.

In a more general sense, the present invention provides for a method of screening the ability of a drug candidate to inhibit the reaction of a plurality of members of a protein family with their substrate, comprising the following steps: combining the drug candidate and the substrate in a fluid sample; delivering the fluid sample to the reactive sites of a device of the present invention, wherein each different member of the protein family is immobilized to a different reactive site; and detecting, either directly or indirectly, for the inhibition of product formation at each reactive site.

To test a drug candidate's ability to interfere with the translocation of a ligand through a lipid bilayer, drug candidate and ligand are delivered in a fluid sample to each microchannel. After a short incubation period the microchannels may be flushed with fluid lacking ligand and any inhibition of passage of the ligand through the lipid bilayer is determined by measuring changes in fluorescence, absorption, or electrical charge.

In an alternative embodiment of the invention, the device of the invention is used to screen a plurality of components, each in separate fluid samples, for their ability to interact with a biological moiety. The method of this embodiment comprises first delivering each of the different fluid samples to separate reactive sites of the invention device, wherein the separate reactive sites of the device each comprise the immobilized biological moiety. The next step comprises detecting, either directly or indirectly, for the interaction of the immobilized biological moiety at each reactive site with the component delivered to that reactive site. Preferably, each of the reactive sites is in a microchannel oriented parallel to microchannels of other reactive sites on the device, wherein the microchannels are microfabricated into or onto the substrate. As before, the interaction being assayed by this method may be any type of interaction normally observed for biological moieties including a catalytic reaction of an enzyme, a binding event, or a translocation by a membrane protein through a lipid bilayer.

One embodiment of the invention provides a method for screening a plurality of different proteins in parallel for their ability to interact with a particular protein, comprising the following steps: delivering different fluid samples, each containing at least one of the different proteins, to separate reactive sites of the device of the invention, wherein the particular protein is immobilized on each of the separate reactive sites; and detecting, either directly or indirectly, for the interaction of the particular protein with the different proteins at each of the reactive sites.

An alternative embodiment of the invention provides for a method for screening a plurality of drug candidates in parallel for their ability to inhibit a reaction of an enzyme with its substrate. This method first involves adding the enzyme's substrate to a plurality of fluid samples, each of which contains at least one of the drug candidates of interest. Next, each of the fluid samples is delivered to a reactive site of the invention device, preferably a microchannel array. In this embodiment, each reactive site of the device features the immobilized enzyme.

Finally, inhibition of product formation at each reactive site (due to the presence of the drug candidate in the solution) is monitored.

Another aspect of the invention provides a method for screening a plurality of binding candidates in parallel for their ability to bind a biological moiety. This method comprises first delivering different fluid samples, each containing at least one of the binding candidates, to the reactive sites of the invention device, wherein the separate reactive sites each comprise the immobilized biological moiety. An optional next step comprises washing the reactive sites with fluid which does not contain the binding candidate in order to elute unbound or nonspecifically bound binding candidates, and detecting, either directly or indirectly, for the presence or amount of said binding candidate retained at each reactive site.

An alternative method for screening a plurality of binding candidates in parallel for their ability to bind a biological moiety is also provided by the present invention comprises the following: adding a known ligand of the biological moiety to a plurality of fluid samples, each of the fluid samples containing at least one of the binding candidates; delivering a different fluid sample to each of the reactive sites of the invention device, wherein the separate reactive sites of the device each comprise the immobilized biological moiety; optionally, washing said reactive sites with fluid that contains neither the known ligand nor a binding candidate in order to elute unbound molecules from each from the reactive sites; detecting the presence of the known ligand retained at each reactive site; and comparing the retention of the known ligand in the presence of the binding candidate with retention of the known ligand in the absence of the binding candidate.

The present invention also provides for a method of pairing a plurality of different proteins with their substrates. In this method, a fluid sample comprising a substrate of a known enzyme family is first delivered to the reactive sites of the invention device where each reactive site of the device comprises a different protein immobilized on the site. Next, any suitable detection means may be used to detect, either directly or indirectly, for the presence of product formed by the

reaction of the substrate with the protein of each reactive site. This method is useful for identifying the function of multitudes of proteins with no known function.

In another aspect of the invention, a method for pairing a plurality of different proteins with their ligands is provided. This method comprises delivering a fluid sample comprising a ligand of a known protein family to the reactive sites of the invention device, wherein each reactive site of the device comprises a different immobilized protein; optionally, washing the reactive sites with fluid that does not contain the ligand to remove unbound ligand from the reactive sites of the device; and detecting, either directly or indirectly, the presence or amount of the ligand retained at each reactive site. This method is useful for identifying to which protein family a protein of unknown function may belong.

Still another embodiment of the invention provides a method for detecting in a fluid sample the presence of a plurality of analytes. The steps of this method comprise delivering the fluid sample to the reactive sites of the invention device, wherein a biological moiety which reacts with at least one of the analytes is immobilized on each of the reactive sites, and detecting for the interaction of the analyte with the immobilized biological moiety at each reactive site.

Another method for detecting in a fluid sample the presence of a plurality of analytes, comprises the following steps: delivering the fluid sample to the reactive sites of the invention device, wherein a biological moiety which binds at least one of the analytes is immobilized on each of the reactive sites; optionally, washing said reactive sites with an analyte-free fluid to remove unbound or nonspecifically bound analyte from each reactive site; and detecting, either directly or indirectly, the presence or amount of analyte retained at each reactive site.

The methods for the parallel detection of a plurality of analytes are applicable to a variety of diagnostic uses. The analytes may optionally be compounds in a body fluid or cellular extract whose presence or amount is

indicative of a disease condition in an organism. In one example, the origin of the analytes may be a pathogen which has infected the organism. Alternatively, the analytes may be expression products of a cell or population of cells in the organism. Such a method can be useful in the evaluation of a tumor or other disease state in a tissue of the organism.

(c) Examples

The following specific examples are intended to illustrate the invention and should not be construed as limiting the scope of the claims:

Example 1. Fabrication of a microchannel array by bulk micromachining.

In a preferred embodiment microchannel arrays are fabricated via standard microstereolithography into the device material (bulk micromachining).

Alternative techniques include surface-micromachining and LIGA (injection molding). Usually, a computer-aided design pattern (reflecting the final channel geometries) is transferred to a photomask using standard techniques, which is then used to transfer the pattern onto a silicon wafer coated with photoresist.

In a typical example, the device ("chip"), with lateral dimensions of 50 x 15 mm, contains a series of 100 parallel channels separated with a spacing of 250  $\mu\text{m}$ . Each channel is 5 mm long and has a cross-section of 100 x 100  $\mu\text{m}$ . The channel volume is 50 nl. 4" diameter Si(100) wafers (Virginia Semiconductor). Si(100) wafers are first cleaned in a 3:1 mixture of  $\text{H}_2\text{SO}_4$ , conc.: 30%  $\text{H}_2\text{O}_2$  (90°C, 10 min), rinsed with deionized water (18 M $\Omega$ cm), finally passivated in 1% aqueous HF, and singed at 150°C for 30 min. After the wafer has been spincoated with polymethyl methacrylate PMMA as positive photoresist and prebaked for 25 minutes at 90 °C, it is exposed using a Karl Suss contact printer and developed according to standard protocols. The wafer is then dried and postbaked at 110 °C for 25 min. Deep silicon reactive ion etching (RIE) is used to anisotropically dry-etch the channel features into the bulk material resulting in high aspect ratio, vertical sidewall features in the silicon (etch rate 2.5  $\mu\text{m}/\text{min}$ ). In the next step,

the wafer is primed with a 20 nm thick titanium layer, followed by a 200 nm thick gold layer both layers deposited using electron-beam evaporation (5 Å/s). After resist stripping (acetone) and a short plasma treatment, the device is covered and sealed with a 50 µm thin glass cover (pyrex 7740) using low-temperature field assisted glass-silicon bonding resulting in a multichannel array with inlet and outlet ports. The gold-coated channel walls can then be further chemically modified to achieve the desired bioreactive and biocompatible properties (see Example 3, below).

Additional details of these procedures can be found in the following references: Madou, *Fundamentals of Microfabrication*, CRC Press (1997); Wolf and Tauber, *Silicon Processing for the VLSI Era, Vol. 1: Process Technology*, Lattice Press, (1986); and Thomson et al., *Introduction to Microlithography*, American Chemical Society, (1994).

Example 2. Fabrication of a microchannel array by sacrificial micromachining.

In sacrificial micromachining, the bulk material is left essentially untouched. Various thick layers of other materials are built up by either physical vapor deposition (PVD), plasma-enhanced chemical vapor deposition (PECVD) or spin coating and selectively remain behind or are removed by subsequent processing steps. Thus, the resulting channel walls are chemically different from the bottom of the channels and the resist material remains as part of the microdevice. Typical resist materials for sacrificial micromachining are silicon nitride ( $\text{Si}_3\text{N}_4$ ), polysilicon, thermally grown silicon oxide and organic resists such as epoxy-based SU-8 and polyimides allowing the formation of high aspect-ratio features with straight sidewalls.

In a typical example, the device ("chip"), with lateral dimensions of 50 x 15 mm, contains a series of 100 parallel channels separated with a spacing of 250 µm. Each channel is 5 mm long and has a cross-section of 100 x 100 µm. The channel volume is 50 nl. 4" diameter Si(100) wafers (Virginia Semiconductor). Si(100) wafers are first cleaned in a 3:1 mixture of  $\text{H}_2\text{SO}_4$ , conc.: 30%  $\text{H}_2\text{O}_2$

(90°C, 10 min), rinsed with deionized water (18 MΩcm), finally passivated in 1% aqueous HF, and singed at 150°C for 30 min. Spincoating of the wafer with EPON SU-8 results in a 100 μm thick film that is exposed similar to Example 1, above, and developed in a propyleneglycol-monomethyletheracetate (PGMEA) solution resulting in a multi-channel structure with high-aspect ratio vertical sidewalls. Deposition of metal films (20 nm Ti, 200 nm Au) is carried out as described in Example 1, above. The device is covered with a 50 μm thin adhesive glass cover. The gold-coated channel walls can then be further chemically modified to achieve the desired bioreactive and biocompatible properties (see Example 3, below).

Additional details on sacrificial micromachining processes can be found in Lorenz, et al., *Proceedings of MME'96 (Micro Mechanics Europe)*, Barcelona, Spain, October 1996, p. 32-35.

Example 3. Synthesis of an aminoreactive monolayer molecule (following the procedure outlined in Wagner et al., *Biophys. J.*, 1996, 70:2052-2066).

*General.* <sup>1</sup>H- and <sup>13</sup>C-NMR spectra are recorded on Bruker instruments (100 to 400 MHz). Chemical shifts (δ) are reported in ppm relative to internal standard ((CH<sub>3</sub>)<sub>4</sub>Si, δ = 0.00 (<sup>1</sup>H- and <sup>13</sup>C-NMR)). FAB-mass spectra are recorded on a VG-SABSEQ instrument (Cs<sup>+</sup>, 20 keV). Transmission infrared spectra are obtained as dispersions in KBr on an FTIR Perkin-Elmer 1600 Series instrument. Thin-layer chromatography (TLC) is performed on precoated silica gel 60 F254 plates (MERCK, Darmstadt, FRG), and detection was done using Cl<sub>2</sub>/toluidine, PdCl<sub>2</sub> and UV-detection under NH<sub>3</sub>-vapor. Medium pressure liquid chromatography (MPLC) is performed on a Labomatic MD-80 (LABOMATIC INSTR. AG, Allschwil, Switzerland) using a Buechi column (460x36 mm; BUECHI, Flawil, Switzerland), filled with silica gel 60 (particle size 15-40 μm) from Merck.

*Synthesis of 11,11'-dithiobis(succinimidylundecanoate) (DSU).* Sodium thiosulfate (55.3 g, 350 mmol) is added to a suspension of 11-bromo-undecanoic acid (92.8 g, 350 mmol) in 50 % aqueous 1,4-dioxane (1000 ml). The mixture is heated at reflux (90 °C) for 2 h until the reaction to the intermediate Bunte salt was complete (clear solution). The oxidation to the corresponding disulfide is carried out *in situ* by adding iodine in portions until the solution retained with a yellow to brown color. The surplus of iodine is retitrated with 15 % sodium pyrosulfite in water. After removal of 1,4-dioxane by rotary evaporation the creamy suspension is filtered to yield product *11,11'-dithiobis(undecanoic acid)*. Recrystallization from ethyl acetate/THF provides a white solid (73.4 g, 96.5 %): mp 94 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub> / CD<sub>3</sub>OD 95 : 5): δ 2.69 (t, 2H, J = 7.3 Hz), 2.29 (t, 2H, J = 7.5 Hz), 1.76-1.57 (m, 4H), and 1.40-1.29 (m, 12H); FAB-MS (Cs<sup>+</sup>, 20 keV): m/z (relative intensity) 434 (100, M<sup>+</sup>). Anal. Calcd. for C<sub>22</sub>H<sub>42</sub>O<sub>4</sub>S<sub>2</sub>: C, 60.79; H, 9.74; S, 14.75. Found: C, 60.95; H, 9.82; S, 14.74. To a solution of *11,11'-dithiobis(undecanoic acid)* (1.0 g, 2.3 mmol) in THF (50 ml) is added N-hydroxysuccinimide (0.575 g, 5 mmol) followed by DCC (1.03 g, 5 mmol) at 0 °C. After the reaction mixture is allowed to warm to 23 °C and is stirred for 36 h at room temperature, the dicyclohexylurea (DCU) is filtered. Removal of the solvent under reduced pressure and recrystallization from acetone/hexane provides *11,11'-dithiobis(succinimidylundecanoate)* as a white solid. Final purification is achieved by medium pressure liquid chromatography (9 bar) using silica gel and a 2:1 mixture of ethyl acetate and hexane. The organic phase is concentrated and dried in vacuum to afford *11,11'-dithiobis(succinimidylundecanoate)* (1.12 g, 78 %): mp 95 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 2.83 (s, 4H), 2.68 (t, 2H, J = 7.3 Hz), 2.60 (t, 2H, J = 7.5 Hz), 1.78-1.63 (m, 4H), and 1.43-1.29 (m, 12H); FAB-MS (Cs<sup>+</sup>, 20 keV): m/z (relative intensity) 514 (100), 628 (86, M<sup>+</sup>). Anal. Calcd. for C<sub>30</sub>H<sub>48</sub>N<sub>2</sub>O<sub>8</sub>S<sub>2</sub>: C, 57.30; H, 7.69; N, 4.45; S, 10.20. Found: C, 57.32; H, 7.60; N, 4.39; S, 10.25.



Example 4. Formation of an aminoreactive monolayer on gold (following the procedure of Wagner et al., *Biophys. J.*, 1996, 70:2052-2066).

Monolayers based on 11,11'-dithiobis(succinimidylundecanoate) (DSU) are deposited on Au(111) surfaces of microdevices described under Examples 1 and 2 by immersing them into a 1 mM solution of DSU in chloroform at room temperature for 1 hour. After rinsing with 10 volumes of solvent, the N-hydroxysuccinimide-terminated monolayer are dried under a stream of nitrogen and immediately used for protein immobilization.

Example 5. Expression and purification of HIV protease variants.

The HIV protease (Genebank HIVHXB2CG) is an essential component of the HIV life cycle, and a major target in anti-viral therapy. HIV protease is required for the proteolytic processing of the gag and gag-pol gene products into functional proteins. Inhibition of HIV protease prevents the production of infectious viral progeny, and hence further rounds of infection. HIV protease belongs to the family of aspartic proteases and is a symmetric homodimer with an active site formed at the interface of the two 99 amino acids long subunits. The core residues in the active site consist of a conserved tripeptide motif (Asp-Thr-Gly) (Roberts et al., *Science*, 1990, 248:358). Resistant variants of HIV protease have emerged against all inhibitors currently used. Most prevalent mutations causing resistance individually or in combination are: L10R, D30N, M46I, L63P, A71V, V82F (Kaplan et al., *Proc. Natl. Acad. Sci.*, 1994, 91:5597; Ho et al., *J. Virol.* 1994, 68: 2016; Condra et al., *Nature*, 1995, 374:569; Schock et al., *J. Biol. Chem.*, 1996, 271:31957; Korant and Rizzo, *Adv. Exp. Med. Biol.*, 1997, 421:279). Additional mutations that preserve protease activity are systematically generated (Loeb et al., *Nature*, 1989, 340:397).

Mutant proteases are generated by PCR mutagenesis (Weiner et al., *Gene*, 1994, 151:119) and expressed in *Escherichia coli* using two approaches: (i) mutant and wild-type protease cDNAs are cloned into a *Escherichia coli* expression vector containing a N-terminal histidine tag (H<sub>6</sub>; Hochuli et al.,

*Biotechnology* 1988, 6:1321) followed by a factor Xa cleavage site, while the stop codon of HIV protease is replaced by a sequence encoding a lysine tag (K<sub>6</sub>) followed by a stop codon. The resulting fusion protein is purified from inclusion bodies as described in Wondrak and Louis, *Biochemistry*, 1996, 35:12957, and the histidine tag removed by factor Xa as described in Wu et al., *Biochemistry*, 1998, 37:4518; or (ii) mutant and wild-type protease cDNAs are cloned into an *Escherichia coli* expression vector creating a fusion between HIV protease, a tri-glycine linker, glutathione S-transferase (GST) and a lysine-tag (HIV-GST-K<sub>6</sub>). The autoprocessing site F\*P at the carboxy terminus of the HIV protease is changed to F\*I to prevent self-cleavage of the fusion proteins (Louis et al., *Eur. J. Biochem.*, 1991, 199:361). The resulting proteins HIV-GST-K<sub>6</sub> are purified from *Escherichia coli* lysates by standard chromatography on glutathione agarose beads and stored in an amine-free buffer at -80°C (25 mM HEPES, pH 7.5, 150 mM NaCl).

Example 6. Immobilization of fusion proteins on an aminoreactive monolayer.

HIV protease variants, in the form of HIV-GST-K<sub>6</sub>, and GST-K<sub>6</sub> are immobilized to the aminoreactive monolayer surface of the microchannel device (see Example 4, above). HIV-GST-K<sub>6</sub> and GST-K<sub>6</sub> are diluted to concentrations of 1 µg/ml in 25 mM HEPES buffer (pH 7.5) containing 150 mM NaCl. First, 50 µl of protein-free buffer is transferred through the channels to hydrate the monolayer surface. After 5 min of incubation, 10 µl of the corresponding protein solutions are flushed through the channels to guarantee total replacement with protein-containing solution. Immobilization is finished after 30 min at room temperature. The channels are rinsed with 50 µl immobilization buffer and subjected to analysis. Each microchannel displays a different HIV-GST-K<sub>6</sub> variant or control (GST-K<sub>6</sub>). Ultrapure water with a resistance of 18 MΩcm is generally used for all aqueous buffers (purified by passage through a Barnstead Nanopure® system).

Example 7. Assay of protease activity in microchannels.

HIV protease requires at least a heptapeptide substrate (Moore et al., *Biochem. Biophys. Res. Commun.*, 1989, 159:420). To analyze the activity of the different HIV variants, a continuous assay based on intra-molecular fluorescence resonance energy transfer (FRET) is used. A peptide substrate corresponding to the p17-p24 cleavage site of the viral gag protein (Skalka, *Cell*, 1989, 56:911) is modified by the addition of an energy-transfer pair (Geoghegan et al., *FEBS Lett.*, 1990, 262:119). In Dns-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Trp (Dns-SSQNYPIVW), the Dns (dansyl) and Trp groups are the N- and C-terminal extensions, respectively (Geoghegan et al.). Excitation of Trp is at 290 nm, and emission of Dns is at 575 nm. Cleavage of the peptide at the Tyr-Pro bond reduces the Dns emission and increases Trp emission at 360 nm. The modified heptapeptide Dns-SSQNYPIVW is prepared as described (Geoghegan et al.) and analyzed by amino acid analysis, nuclear magnetic resonance and mass spectrometry. The purity is checked by HPLC analysis using a Vydac C-4 column and an acetonitrile gradient in 0.1% TFA. In order to test the activity of all the HIV variants described above, each microchannel with an immobilized HIV variant (see Example 6) is filled with 20  $\mu$ M of Dns-SSQNYPIVW in 50 mM sodium acetate, pH 5.5, 13 % glycerol, 10 mM DTT. Addition of the substrate to the immobilized proteins leads to time-dependent intensity changes in the fluorescence emission spectrum. The 360 nm Trp emission peak progressively will increase to about 2.5 times its initial intensity, while the Dns group's emission band (575 nm) will decline in intensity. This intensity change will be observed in all the channels containing active forms of the HIV variants. To control for changes in background fluorescence, GST-K<sub>6</sub> fusion protein is measured in parallel.

Competition assays can be carried out to test the specificity of the proteolysis by the HIV variants. In one assay, both Dns-SSQNYPIVW and a small organic molecule that is to be tested for its potential as a drug, is delivered in a 50 mM sodium acetate, pH 5.5, 13 % glycerol, 10 mM DTT solution to each

channel. An organic molecule which acts as an inhibitor for a wide range of HIV protease variants will diminish the Trp emission peak increase and the Dns emission band decrease associated with reaction of the protease with the peptide substrate in a number of the microchannels. A less desirable drug candidate, on the other hand, will inhibit the reaction of the HIV protease with the peptide substrate only in selected microchannels (or none at all).

Protease inhibitors Sequinavir (Roche), Ritonavir (Abbot) or Indinavir (Merck) can also be added to the reaction buffer and used as positive controls for the specificity of inhibition. Sequinavir will inhibit all the HIV variants except those containing either the G48V or the L90M mutation. Ritonavir in contrast is unable to block the activity of the M46I, L63P, A71V, V82F and the I84V variants. Indinavir has a similar inhibition pattern like Ritonavir except that the A71V variant is not affected. In addition Indinavir is not able to decrease the activity of the L10R HIV protease variant.

These experiments demonstrate how an HIV-variant microchannel device may be used to test the inhibitory effect of small organic molecules on the activity of the HIV protease.

All documents cited in the above specification are herein incorporated by reference. Various modifications and variations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the following claims.

What is claimed is:

1. A device for analyzing components of a fluid sample, having a plurality of noncontiguous reactive sites, each of said sites comprising:
  - (a) a substrate;
  - (b) an organic thinfilm chemisorbed or physisorbed on a portion of the surface of said substrate; and
  - (c) a biological moiety immobilized on said organic thinfilm;wherein each of said sites may independently react with a component of the fluid sample and are separated from each other by a region of said substrate that is free of said organic thinfilm.
2. The device of Claim 1, further comprising an affinity tag, wherein said biological moiety is immobilized to said organic thinfilm by said affinity tag.
3. The device of Claim 1, wherein the organic thinfilm is less than about 20 nm thick.
4. The device of Claim 1, wherein said organic thinfilm comprises a monolayer.
5. The device of Claim 1, wherein the monolayer comprises a self-assembled monolayer comprising molecules of the formula
$$(X)_aR(Y)_b$$
wherein R is a spacer, X is a functional group that binds R to the surface, Y is a functional group for binding the biological moiety onto the monolayer, and a and b are, independently, integers.
6. The device of Claim 5, wherein both a and b are 1.

7. The device of Claim 5, wherein:  
said substrate is selected from the group consisting of silicon, silicon dioxide, indium tin oxide, alumina, glass, and titania; and  
X, prior to incorporation into said monolayer, is selected from the group consisting of a monohalosilane, dihalosilane, trihalosilane, trichlorosilane, trialkoxysilane, dialkoxysilane, monoalkoxysilane, carboxylic acid, and phosphate.
8. The device of Claim 5, wherein the substrate comprises silicon and X is an olefin.
9. The device of Claim 1, wherein the substrate comprises a polymer.
10. The device of Claim 5, further comprising at least one coating between said substrate and said monolayer, wherein said coating is formed on the substrate or applied to the substrate.
11. The device of Claim 10, wherein:  
said coating comprises a noble metal film; and  
X, prior to incorporation into said monolayer, is a functional group selected from the group consisting of an asymmetrical or symmetrical disulfide, sulfide, diselenide, selenide, thiol, isonitrile, selenol, trivalent phosphorus compounds, isothiocyanate, isocyanate, xanthanate, thiocarbamate, phosphines, amines, thio acid and dithio acid.
12. The device of Claim 10, wherein the coating comprises titania or tantalum oxide and X is a phosphate group.
13. The device of Claim 2, further comprising an adaptor that links the affinity tag to the immobilized biological moiety.

14. The device of Claim 1 which comprises at least about 10 reactive sites.
15. The device of Claim 13 which comprises at least about 100 reactive sites.
16. The device of Claim 1 which comprises at least about 10 different immobilized biological moieties.
17. The device of Claim 16 which comprises at least about 100 different immobilized biological moieties.
18. The device of Claim 1, wherein all of the biological moieties on the reactive sites are functionally related.
19. The device of Claim 1, wherein all of the biological moieties on the reactive sites are structurally related.
20. The device of Claim 1, wherein the biological moiety is a polynucleotide.
21. The device of Claim 1, wherein the biological moiety is a protein.
22. The device of Claim 21, wherein all of the biological moieties on the reactive sites are members of the same protein family.
23. The device of Claim 22, wherein the protein family is selected from the group consisting of growth factor receptors, hormone receptors, neurotransmitter receptors, catecholamine receptors, amino acid derivative receptors, cytokine receptors, extracellular matrix receptors, antibodies, lectins, cytokines, serpins, proteases, kinases, phosphatases, ras-like GTPases, hydrolases, steroid hormone receptors, transcription factors, heat-shock transcription factors. DNA-binding

proteins, zinc-finger proteins, leucine-zipper proteins, homeodomain proteins, intracellular signal transduction modulators and effectors, apoptosis-related factors, DNA synthesis factors, DNA repair factors, DNA recombination factors, cell-surface antigens, hepatitis C virus (HCV) proteases and HIV proteases.

24. The device of Claim 21, wherein the biological moiety is an antibody or an antibody fragment.

25. The device of Claim 1, wherein the biological moiety is a protein-capture agent.

26. The device of Claim 1, wherein said device comprises a micromachined or microfabricated device.

27. The device of Claim 1, wherein each of said reactive sites is in a microchannel oriented parallel to microchannels of other reactive sites on the device, wherein said microchannels are microfabricated into or onto said substrate.

28. The device of Claim 27, wherein said device comprises at least about 10 microchannels.

29. The device of Claim 28, wherein said device comprises from about 100 to about 500 microchannels.

30. The device of Claim 27, wherein said device comprises from about 2 to about 500 parallel microchannels per  $\text{cm}^2$ .

31. The device of Claim 27, further comprising a cover over the microchannels.



32. The device of Claim 31, wherein the volume of said microchannel is between about 5 nanoliters and about 300 nanoliters.
33. The device of Claim 32, wherein the volume of said microchannel is between about 10 nanoliters and about 50 nanoliters.
34. The device of Claim 27, wherein the width and depth of said microchannel each are between about 10  $\mu\text{m}$  and about 500  $\mu\text{m}$ .
35. A method for screening a plurality of different biological moieties in parallel for their ability to interact with a component of a fluid sample, comprising:
- (a) delivering the fluid sample to the reactive sites of a device of Claim 1, wherein each of the different biological moieties is immobilized on a different reactive site of the device; and
  - (b) detecting, either directly or indirectly, the interaction of said component with the immobilized biological moiety at each reactive site.
36. A method for screening a plurality of different biological moieties in parallel for their ability to react with a component of a fluid sample, comprising:
- (a) delivering the fluid sample to the reactive sites of a device of Claim 1, wherein each of the different biological moieties is immobilized on a different reactive site of the device; and
  - (b) detecting, either directly or indirectly, formation of product of the reaction of said component with the immobilized biological moiety at each reactive site.
37. A method for screening a plurality of biological moieties in parallel for their ability to bind a component of a fluid sample, comprising:

(a) delivering said fluid sample to the reactive sites of a device of Claim 1, wherein each different biological moiety is immobilized on a different reactive site of the device; and

(b) detecting, either directly or indirectly, the presence or amount of said component retained at each reactive site.

38. A method for screening a plurality of components in separate fluid samples for their ability to interact with a biological moiety, comprising:

(a) delivering each of the different fluid samples to separate reactive sites of the device of Claim 1, wherein the separate reactive sites of the device each comprise the immobilized biological moiety; and

(b) detecting, either directly or indirectly, for the interaction of the immobilized biological moiety at each reactive site with the component delivered to that reactive site.

39. A method for screening a plurality of binding candidates in parallel for their ability to bind a biological moiety, comprising:

(a) delivering different fluid samples, each containing at least one of the binding candidates, to separate reactive sites of the device of Claim 1, wherein the separate reactive sites each comprise the immobilized biological moiety; and

(b) detecting, either directly or indirectly, for the presence or amount of said binding candidate retained at each reactive site.

40. A method for screening a plurality of different proteins in parallel for their ability to interact with a particular protein, comprising:

(a) delivering different fluid samples, each containing at least one of the different proteins, to separate reactive sites of the device of Claim 1, wherein the particular protein is immobilized on each of the separate reactive sites; and

(b) detecting, either directly or indirectly, for the interaction of the particular protein with the different proteins at each of the reactive sites.

41. A method for pairing a plurality of proteins with their substrates, comprising:
- (a) delivering a fluid sample comprising a substrate of a known enzyme family to the reactive sites of a device of Claim 1, wherein each reactive site of the device comprises a different immobilized protein; and
  - (b) detecting, either directly or indirectly, for product formed by the reaction of the substrate with the immobilized protein of each reactive site.
42. A method for pairing a plurality of proteins with their ligands, comprising:
- (a) delivering a fluid sample comprising a ligand of a known protein family to the reactive sites of a device of Claim 1, wherein each reactive site of the device comprises a different protein; and
  - (b) detecting, either directly or indirectly, for the presence or amount of the ligand retained at each reactive site.
43. A method for detecting in a fluid sample the presence of a plurality of analytes, comprising:
- (a) delivering the fluid sample to the reactive sites of a device of Claim 1, wherein a biological moiety which reacts with one of said analytes is immobilized on each of the reactive sites; and
  - (b) detecting for the interaction of the analyte with the immobilized biological moiety at each reactive site.
44. A method for detecting in a fluid sample the presence of a plurality of analytes, comprising:
- (a) delivering the fluid sample to the reactive sites of a device of Claim 1, wherein a biological moiety which binds one of said analytes is immobilized on each of the reactive sites; and

(b) detecting, either directly or indirectly, for the presence of analyte retained at each reactive site.

45. A device for analyzing components of a fluid sample, comprising:

- (a) a substrate;
- (b) a plurality of parallel microchannels microfabricated into or onto said substrate; and
- (c) a biological moiety immobilized within at least one of said parallel microchannels, wherein said biological moiety may interact with a component of the fluid sample.

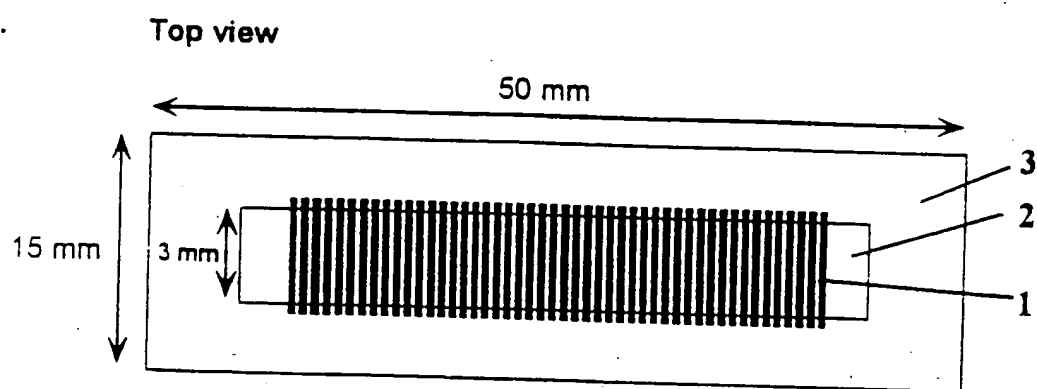


Fig. 1

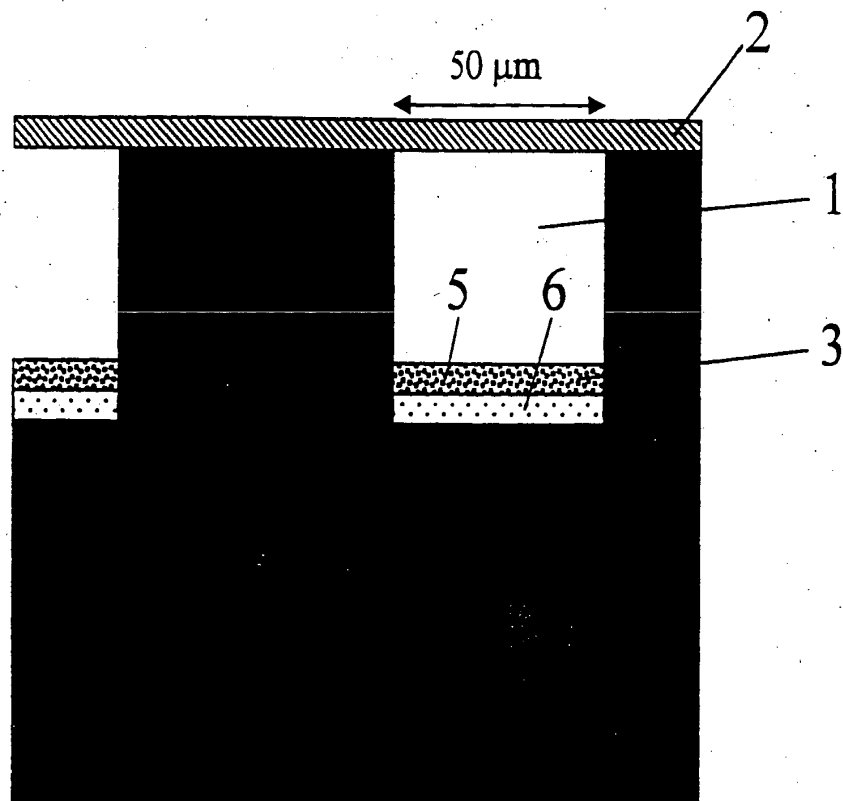


Fig. 2

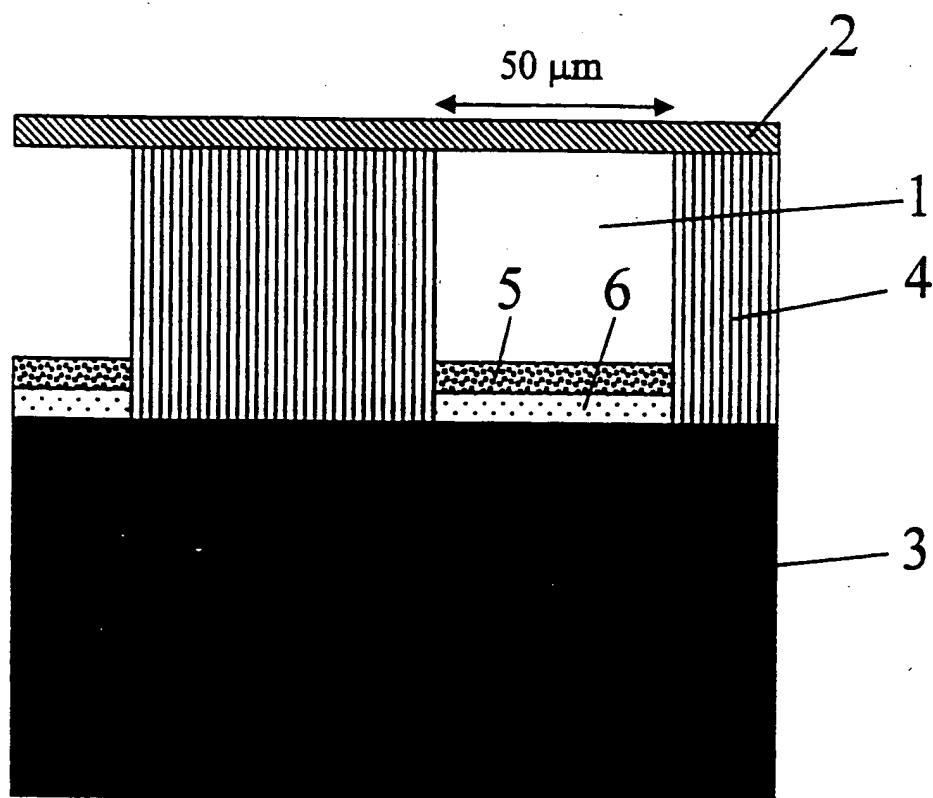


Fig. 3

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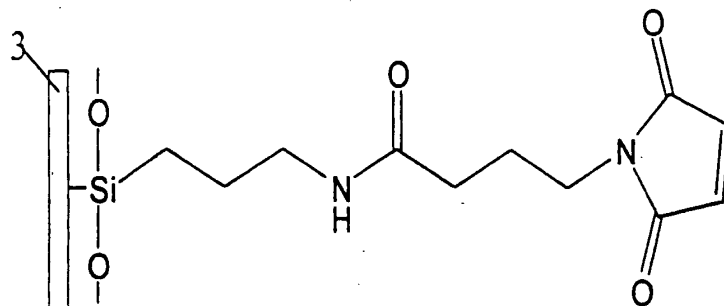


Fig. 4

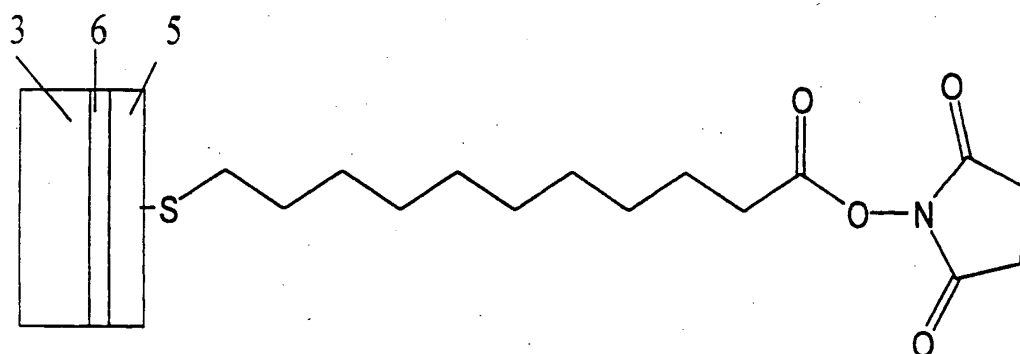


Fig. 5



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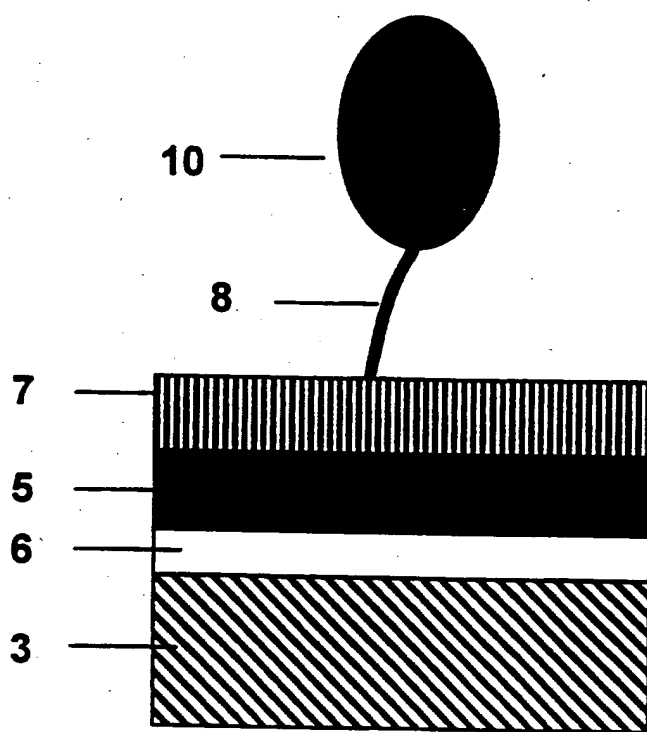


Fig. 6

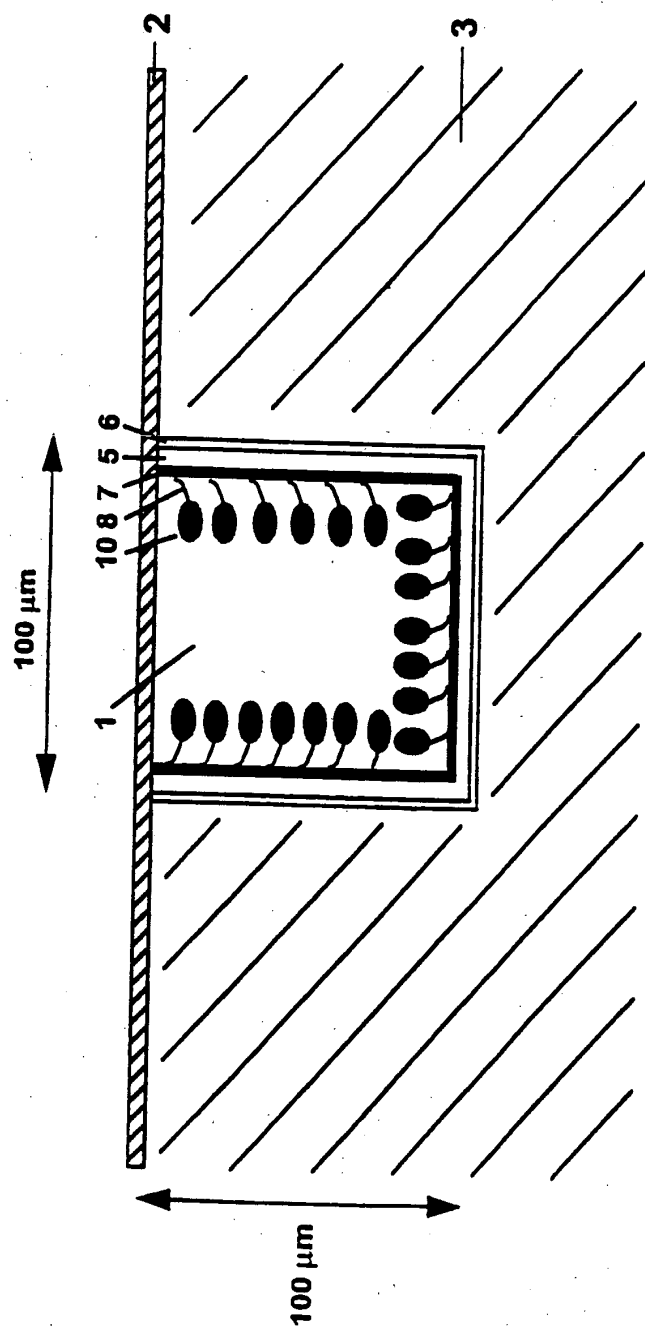


Fig. 7

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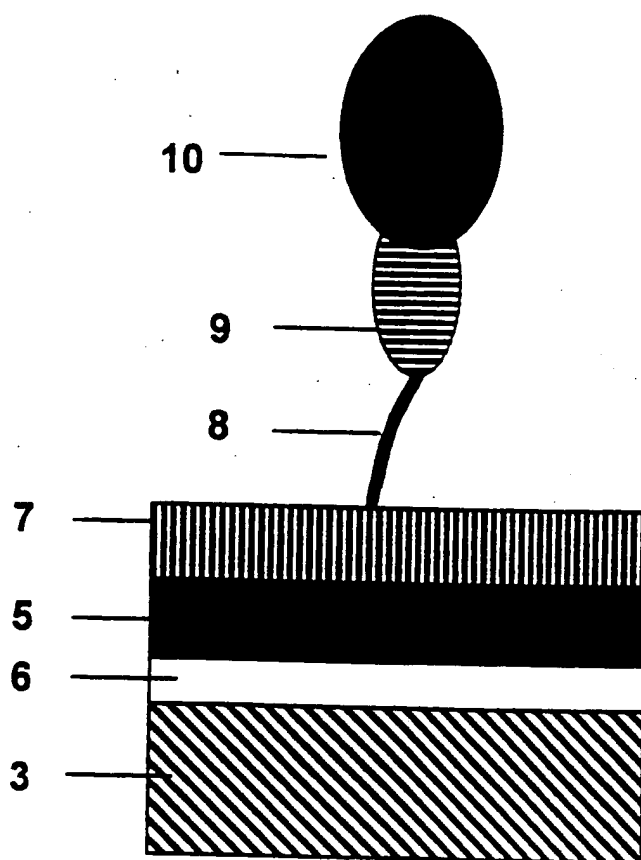


Fig. 8

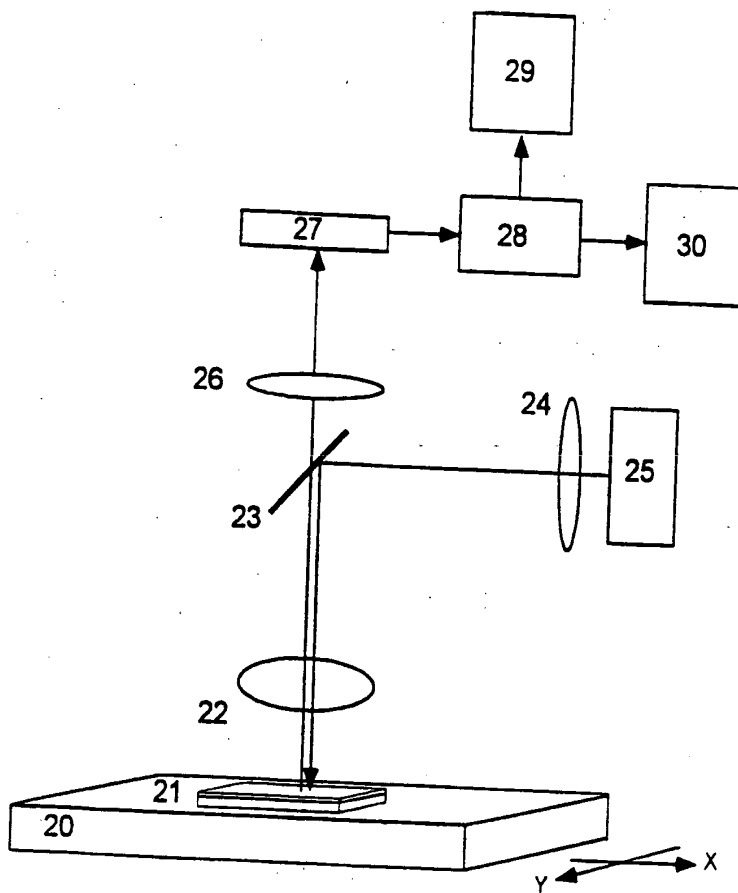


Fig. 9

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: UPA, A UNIVERSAL PROTEIN ARRAY SYSTEM		

## (57) Abstract

This invention relates to ordered arrays of molecules, for instance polypeptides or proteins. Such arrays, referred to as universal protein arrays, are described in both macro- and microarray formats. Methods of production and use of such arrays are also described. Also disclosed are kits for the use of such arrays and the preparation of probes for use with them.

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## UPA, A UNIVERSAL PROTEIN ARRAY SYSTEM

### FIELD

5       The present invention relates to detection of interactions between polypeptide and protein, DNA, RNA and/or ligand molecules.

### BACKGROUND

10       Gene expression in eukaryotic cells is controlled by numerous fundamental and selective protein-protein, protein-DNA, protein-RNA and protein-ligand interactions. Cancer, as well as other genetic diseases, results from abnormal gene expression. Interactions of proteins with proteins and other biomolecules play a pivotal role in almost every aspect of gene expression. Therefore, factors involved in these interactions, including transcription factors, signal transduction factors, growth factors and the products of other oncogenes, tumor suppressor genes, viral genes and many cellular  
15       genes, have been implicated as potential targets for new drugs (Hurst, *Eur. J. Cancer*, 32A, 1857-1863, 1996; Bustin and McKay, *Br. J. Biomed. Sci.*, 51, 147-157, 1994; Powis, *Pharmac. Ther.*, 62, 57-95, 1994; Krantz, *Nature Biotechnol.*, 16, 1294, 1998).

      Use of transcription factors has proved to be a successful means to identify new drug targets in cancer and other human disease. The basal transcription machinery of class II genes consists of at  
20       least six general transcription factors, including TFIIB, TFIID, TFIIE, TFIIIF, TFIIH and RNA polymerase II. However, an additional activator(s) and coactivator(s) are required for regulated (activated) transcription (Orphanides *et al.*, *Genes Dev.*, 10, 2657-2683, 1996; Ptashne and Gann, *Nature*, 386, 569-577, 1997). Both basal and activated transcriptions are controlled largely through protein-protein interactions between transcription factors and through protein-DNA interactions.  
25       Thus, insight into factor communication holds not only the key to understanding mechanisms of gene regulation, but also provides a means of understanding mechanisms of pathogenesis and of identifying anticancer drugs.

      At present, in addition to the two-hybrid system and co-immunoprecipitation assays usually used to detect protein-protein interactions *in vivo*, the glutathione S-transferase (GST) pull-down  
30       assay is one of the more common methods to determine specific protein-protein interactions *in vitro*. Cross-linking, gel mobility shift, footprinting and others have been often used to study protein-DNA and protein-RNA interactions (Fields and Sternglanz, *Trends Genet.*, 10, 286-292, 1994; Harris, *Methods Mol. Biol.*, 88, 87-99, 1998). Recently, several methods, including serial analysis of gene expression (SAGE) (Velculescu *et al.*, *Science*, 270, 484-487, 1995), cDNA microarrays (Skena *et al.*, *Science*, 270, 467-470, 1995) and oligonucleotide-based DNA chips (Chee *et al.*, *Science*, 274, 610-614, 1996), have been employed to study the relationship between gene expression and cancer  
35

and have made significant contributions to our understanding of the mechanism of tumorigenesis. However, knowledge of which *trans*-acting factors are involved and how they change gene expression patterns is still limiting due to the lack of efficient and reproducible techniques to examine intermolecular communications.

5 Therefore, there still exists a strong need for reliable, simple systems for the detection of interactions of various molecules with proteins of interest.

### SUMMARY

The present invention is a high-throughput, parallel-analysis method (generally referred to as a universal protein array (UPA) system) that can be used effectively and quantitatively to determine polypeptide interactions with other molecules, for instance biomolecules. UPA can be used in molecular biology and biochemistry laboratories to study protein-protein, protein-DNA, protein-RNA and protein-ligand interactions, for instance those involved in gene expression pathways, including transcription, RNA processing, replication, translation, signal transduction and others. UPA can also be used to screen compounds to test their possible efficacy as new drugs based on their ability to bind to polypeptides or block binding of other molecules to polypeptides.

15 This invention provides arrays, particularly universal protein arrays. Such arrays have a plurality of target polypeptide samples bound to a solid support. The arrays will include at least 10 polypeptide samples, which can be arranged in any addressable pattern including a grid or radial pattern. These sample polypeptides may be immobilized on the solid support. In some embodiments, only one polypeptide is arrayed at each address.

In certain embodiments of the invention, the sample target polypeptides used in the array are substantially pure. A preparation of substantially pure polypeptide for use in the arrays of this invention may be purified such that the desired protein represents at least 50% of the total protein content of the preparation. In other embodiments, a substantially pure protein will represent at least 25 60%, at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% or more of the total protein content of the preparation.

Arrays of this invention include macro- and microarrays, or combinations thereof. In these arrays, the polypeptide samples can be supported on any solid support, for instance glass, nitrocellulose, polyvinylidene fluoride, nylon, fiber, or combinations thereof. In particular, 30 embodiments, the support is a glass slide. This slide may additionally have a polymerized layer attached or associated with at least one surface (e.g., face) to provide a specific region for immobilization of the target polypeptides.

Particular arrays of the invention contain polypeptides that are related to each other in at least one way or share some common characteristic. Certain arrays, for instance, contain 35 polypeptides that are transcriptional factors, transcriptional activators, and/or transcriptional coactivators. Specific examples of such transcription-related arrays will include polypeptides chosen

from the following group of polypeptides: TFIIA, TFIIB, TBP, f:TFIID, TFIIE, TFIIIF, f:TFIIH, Pol II, RXR, TR, Oct1, Sp1, G4-94, G4-147, G4-AH, G4-VP16, G4-CTF, G4-Sp1, G4-E1A, G4-IE, G4-Tat, PC4-P, PC4-N, PC4-C, PC4-ΔS, PC4-m1, PC4-m2, PC4-m3, PC4-m4, PC4-m5, PC4-m6, PC4-m7, PC4-wt, p52, p75, p75-C, p300-C, PCAF, PCAF-C, TAF250, Topo I (wt), Topo I (mt), Topo I (wt)\*, Topo I (nati), ASF, SR, GST-Nu, and GST-K. This represents specific but non-limiting  
5 examples of certain proteins that may be presented as targets on an array of this invention.

The present invention also provides assays employing these arrays.

Certain embodiments of the invention are array-based protein interaction assays, wherein an array (either a macro- or microarray) of target polypeptide molecules is contacted with a detectable  
10 probe molecule under conditions sufficient to produce binding (*e.g.*, a binding pattern). Binding can then be detected. In certain embodiments, the polypeptides of the array are substantially pure preparations of polypeptide. Polypeptides may for example be stably associated with the surface of the array, which may be a solid support. Examples of such assays include a further step of removing unbound probe molecule(s) prior to detecting the binding pattern of the probe.

15 Probes for use with assays of this invention can be any molecules that might bind to a polypeptide. Examples of probes include single-stranded nucleic acids (DNA or RNA), double-stranded nucleic acids (DNA or RNA), proteins, and ligands (*e.g.*, drugs, toxins, venoms, hormones, co-factors, substrates or reaction products of enzymatic reactions or analogs thereof, transition state analogs, minerals, and so forth). Such probes are detectable, either due to inherent features of the  
20 probe (such as immunogenicity, which can be detected through interaction with an antibody) or through the attachment or association of a label or tag molecule. Examples of tags include fluorescent tags, luminescent tags, and immunogenic tags.

Other assays provided by the invention can be used to determine one or more polypeptide-binding characteristics of a probe molecule. Such assays may include preparing a labeled sample of  
25 the probe molecule (for instance, a nucleic acid molecule, polypeptide or ligand). The probe is contacted to an array of target polypeptides to produce a binding pattern, which can then be detected. In certain embodiments, unbound probe is washed or otherwise removed from the array, for instance prior to detecting the binding pattern, to reduce or remove background signals. Target polypeptides of the arrays used in these assays are stably associated with a solid support.

30 Examples of labels for use with any of the assays of the invention include all labels that can be attached to a probe molecule to facilitate detection of the molecule. Such labels include tags that can be directly detected (*e.g.*, radioisotopes, fluorescent or luminescent tags) as well as labels that require secondary detection (*e.g.*, immunogenic or epitope tags, members of the strept/avidin:biotin system). Probes can also be detectable in the sense that they can be detected based on a characteristic  
35 inherent in the probe itself (*e.g.*, immunogenicity, inherent fluorescence, etc.).

This invention also provides kits for labeling probe molecules to be used with array-based protein interaction assays (*e.g.*, universal protein array based assays). Such kits include at least a tag

capable of being linked to a probe molecule, and instructions for how to use the tag to label probes. Buffers for use in the probe labeling process, or for use in performing the array-based protein assay, may also be provided in the kits. A probe molecule standard (either labeled or unlabeled) may also be included in the kit.

5 Certain probe labeling kits will also include one or more arrays, for instance an array of substantially pure polypeptide molecules.

Other kits provided in this invention are used for determining one or more polypeptide-binding characteristics of a probe molecule. Such kits include a polypeptide array and instructions for its use in determining binding characteristics of at least one probe molecule. The target  
10 polypeptides on these arrays can be substantially pure polypeptide samples, and may be arranged for instance in a grid-like or radial arrangement. Arrays provided in kits can be macro- or microarrays, or both, depending on the specific embodiment of the invention. Buffers for use in the probe labeling process, or for use in performing the array-based protein assay, may also be provided in the kits. One or more probe molecule standards (either labeled or unlabeled) may also be included in the  
15 kit.

Other embodiments of the invention are methods of analyzing proteins, particularly protein-molecule interactions and/or binding characteristics. Certain of these methods include obtaining more than one (a plurality) substantially pure protein specimen, placing a sample of each specimen in an addressable location on a recipient array; and probing the array of specimens with a detectable  
20 probe molecule. Arrays for use in these methods can be macro- or microarray, or combinations thereof. Probe molecules used to assay arrays in these methods can be any molecule, for example a nucleic acid, a polypeptide, a ligand, a fragment thereof, or mixtures thereof.

Other methods provided include methods of analyzing a plurality of binding characteristics of an array of polypeptide samples. In such methods, an array of polypeptide samples is probed at  
25 least twice, sequentially, with at least a first and a second (different) probe molecule. The array may be stripped of bound first probe prior to being assayed with the second probe. Binding patterns for the first and second probes can be detected and analyzed to determine which polypeptides each probe binds to, thereby revealing multiple binding characteristics of the array of polypeptide samples. Arrays used in these methods can be macro- or microarrays, and will include a plurality of target  
30 polypeptide samples (which may be substantially pure) immobilized on a solid support in an addressable pattern. In these methods, the first and second (and so forth) probes can be from any class of molecules.

The foregoing and other features and advantages of the invention will become more apparent from the following detailed description of several embodiments, which proceeds with  
35 reference to the accompanying figures.

## BRIEF DESCRIPTION OF THE FIGURES

### Figure 1: Protein-Protein Interactions

The universal protein array (UPA) provides quantitative detection of specific protein-protein interactions at different salt wash stringencies. Fig. 1A shows the autoradiographic signals detected from the herein-described UPA that was incubated with  $^{32}\text{P}$ -labeled GST-K-p52, then washed with low salt buffer A100 (100 mM KCl) to remove unbound probe, as described in Example 3. Fig. 1B shows the autoradiographic signals detected from the same UPA after it was washed in high salt A1000 buffer (100 mM KCl).

Table 1 is the polypeptide target arrangement key for the array shown in Fig. 1A and 1B.

Fig. 1C is a pictorial representation of the relative affinities of the 48 arrayed proteins for the transcriptional cofactor p52 after the array was washed with buffer A1000 (100 mM KCl). The units are reading units from a densitometer.

### Figure 2: Protein-DNA, Protein-RNA, and Protein-Ligand Interactions

The universal protein array also permits autoradiographic detection of protein-dsDNA (Fig. 2A), protein-ssDNA (Fig. 2B), protein-RNA (Fig. 2C), and protein-ligand (Fig. 2D) interactions. The same UPA was probed with  $^{32}\text{P}$ -labeled nucleic acids (Examples 4 and 5) or with  $^{125}\text{I}$ -labeled ligand (Example 6) as described in the text. Between each application of probe, the UPA was stripped and equilibrated in buffer A100, as described in Example 2.

As for Fig. 1, Table 1 contains the polypeptide target arrangement key for the array shown in Figure 2.

### Figure 3: Detection of ASF/SF2-Interacting Proteins Using a UPA

Sixteen selected proteins/protein fractions were analyzed for interaction with  $^{32}\text{P}$ -labeled 6H(K)ASF/SF2. Fig. 3A shows the key grid of 16 proteins that were arrayed (in a 4 by 4 grid format). Fig. 3B and Fig. 3C are autoradiographs of the binding patterns on the UPA after washing with 100 mM KCl or 500 mM KCl, respectively.

Key to the abbreviations in Fig. 3A: CTD, the C-terminal domain of RNA polymerase II fused to GST; RPB5, RPB6, RPB8, RPB10 $\alpha$  and RPB10 $\beta$  correspond to individual subunits of RNA polymerase II fused to GST; TBP, TATA-binding protein; f.TFIID, affinity-purified flag-tagged TBP-containing TFIID complex from HeLa cells; RXR, retinoid-X receptor; TR, thyroid hormone receptor; His-H1, histone H1; Co-His, co-histones; HMG1, high mobility group protein 1; ASF, alternative splicing factor; GST-Nu, GST-nucleolin fusion; GST-K, GST fused with a synthetic heart muscle kinase site.

## DETAILED DESCRIPTION

## I. Abbreviations and Definitions

## A. Abbreviations

- 5 ASF: alternative splicing factor  
 Co-His: co-histones  
 CTD: the C-terminal domain of RNA polymerase II fused to GST  
 f:TFIID: flag-tagged TBP-containing TFIID complex from HeLa cells  
 G4-94, G4-147, G4-AH, G4-VP16, G4-CTF, G4-Sp1, G4-E1A, G4-IE, G4-Tat: Gal 4 fused to  
 10 different transcription activation domains (see Table 2)  
 GST: Glutathione S-transferase  
 GST-K: GST fused with a synthetic heart muscle kinase site  
 GST-Nu: GST-nucleolin fusion  
 His-H1: histone H1  
 15 HMG1: high mobility group protein 1  
 Oct 1: B-cell specific activator  
 p52: novel transcription factor p52  
 p75: novel transcription factor p75  
 p75-C: C-terminal region of novel transcription factor p75  
 20 p300-C: transcriptional activator  
 PC4: positive cofactor 4  
 PC4-P, PC4-N, PC4-C, PC4-ΔS, PC4-m1, PC4-m2, PC4-m3, PC4-m4, PC4-m5, PC4-m6, PC4-  
 m7, PC4-wt: various PC4 polypeptides (see Table 2)  
 PCAF: a p300/CBP-associated factor that functions as a histone  
 25 PCAF-C: C-terminal region of PCAF  
 Pol II: polymerase II  
 Sp1: class II gene activator  
 SR: serine-arginine protein fraction prepared from HeLa cell nuclear extracts  
 RPB5, RPB6, RPB8, RPB10α and RPB10β: correspond to individual subunits of RNA polymerase  
 30 II fused to GST  
 RXR: retinoid-X receptor  
 TAF250: transcriptional coactivator  
 TBP: TATA-binding protein  
 TFIIA, TFIIB, TBP, f:TFIID, TFIIE, TFIIIF, f:TFIIH: Class II transcription factors IIA, IIB, IID,  
 35 IIE, IIF, and IIH  
 Topo I (wt), Topo I (mt), Topo I (wt)\*, Topo I (nati): various topoisomerase I polypeptides (see  
 Table 2)

TR: thyroid hormone receptor

UPA: universal protein array

### B. Definitions

5 Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes VII*, published by Oxford University Press, 2000 (ISBN 0-19-899276-X); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk*  
10 *Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the invention, the following definition of terms is provided:

15 **Array:** An arrangement of molecules, particularly biological macromolecules (such as polypeptides or nucleic acids) in addressable locations on a substrate. A "microarray" is an array that is miniaturized so as to require microscopic examination for evaluation.

Within an array, each arrayed molecule is addressable, in that its location can be reliably and consistently determined within the at least two dimensions of the array surface. Thus, in ordered arrays the location of each molecule sample is assigned to the sample at the time when it is spotted  
20 onto the array surface and usually a key is provided in order to correlate each location with the appropriate target. Often, ordered arrays are arranged in a symmetrical grid pattern, but samples could be arranged in other patterns (e.g., in radially distributed lines or ordered clusters).

The shape of the sample application "spot" is immaterial to the invention. Thus, though the term "spot" is used throughout this specification, it refers generally to a localized deposit of sample  
25 target polypeptide, and is not limited to a round or substantially round region. For instance, essentially square regions of polypeptide application can be used with arrays of this invention, as can be regions that are essentially rectangular (such as slot blot application), or triangular, oval, or irregular. The shape of the array itself is also immaterial to the invention, though it is usually substantially flat and may be rectangular or square in general shape.

30 A key to one example array is shown in Table 1. Construction of this array is described in Example 1. This array has 48 addresses (individual spots on the array), which are arranged in an 8 by 12 grid, with eight columns labeled "a" through "h" and twelve rows labeled "1" through "12." Each address position can be referred to by a row and column label (e.g., address "1a" in the upper left corner of the array contains transcription factor IIA, abbreviated "TFIIA").

35 In this particular example array, as described below in Example 1, each target polypeptide has been spotted onto the array twice to provide internal controls. The duplicate samples are found in a pair of horizontally adjacent addresses of the array: for instance, transcription factor IIA (TFIIA)

is found at both address 1a and address 1b, collectively addresses 1a/b. This pair of addresses (which contain samples of the same polypeptide) can additionally be referred to by a single number that corresponds to the protein in that pair of addresses. Thus, TFIIA (at addresses 1a and 1b) can also be referred to by the numeral (1) (found centered above addresses 1a and 1b of Table 1). Likewise, the numeral (2) refers to addresses 1c and 1d (1c/d), and designates the two address that contain a sample of transcription factor IIB (TFIIB). Horizontally arranged pairs of addresses containing samples of the same polypeptide are numbered this way through out this particular array, from (1) (referring to TFIIA, in addresses 1a/b) through (48) (referring to GST-K, in addresses 12g/h). These reference numerals (1) through (48) are used in the first column of Table 2 to correlate the binding data discussed in some of the Examples below to the array position key.

**Binding or interaction:** An association between two substances or molecules. The arrays of this invention are used to detect binding of a probe molecule to one or more polypeptides of the array. A probe "binds" to a polypeptide of an array of this invention if, after incubation of the probe (usually in solution or suspension) with or on the array for a period of time (usually 5 minutes or more, for instance 10 minutes, 20 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes or more), a detectable amount of the probe associates with a polypeptide of the array to such an extent that it is not removed by being washed with a relatively low stringency buffer (e.g., 100 mM KCl). Washing can be carried out, for instance, at room temperature, but other temperatures (either higher or lower) can also be used. Probes will bind different polypeptides to different extents, and the term "bind" encompasses both relatively weak and relatively strong interactions. Thus, some binding will persist after the array is washed in a higher salt buffer (e.g., 500 mM or 1000 mM KCl).

The term "binding characteristics of an array for a particular probe" refers to the specific binding pattern that forms between the probe and the array after excess (unbound or not specifically bound) probe is washed away. This pattern (which may contain no positive signals, some or all positive signals, and will likely have signals of differing intensity) conveys information about the binding affinity of that probe for the polypeptides of the array, and can be de-coded by reference to the key of the array (which lists the addresses of the polypeptides on the array surface). The relative intensity of the binding signals from individual polypeptide spots is indicative of the relative affinities of the probe for those polypeptide molecules (assuming that the same number of probe binding sites are immobilized at each address on the array). Quantification of the binding pattern of an array/probe combination can be carried out using any of several existing techniques, including scanning the signals into a computer for calculation of relative density of each spot.

**DNA (deoxyribonucleic acid):** DNA is a long chain polymer that contains the genetic material of most living organisms (the genes of some viruses are made of ribonucleic acid (RNA)). The repeating units in DNA polymers are four different nucleotides, each of which includes one of the four bases (adenine, guanine, cytosine and thymine) bound to a deoxyribose sugar to which a phosphate group is attached. Triplets of nucleotides (referred to as codons) code for each amino acid



in a polypeptide, or for a stop signal. The term "codon" is also used for the corresponding (and complementary) sequences of three nucleotides in the mRNA into which the DNA sequence is transcribed.

5       **High throughput genomics:** Application of genomic or genetic data or analysis techniques that use microarrays or other genomic technologies to rapidly identify large numbers of genes or proteins, or distinguish their structure, expression or function from normal or abnormal cells or tissues.

10       **Isolated:** An "isolated" biological component (such as a nucleic acid molecule, protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, *i.e.*, other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

15       **Nucleic acid:** A deoxyribonucleotide or ribonucleotide polymer in either single or double stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides.

20       **Oligonucleotide:** A linear polynucleotide sequence of up to about 300 nucleotide bases in length, for example a polynucleotide (such as DNA or RNA) which is at least 6 nucleotides, for example at least 15, 50, 100 or even 200 nucleotides long.

25       An oligonucleotide analog refers to moieties that function similarly to oligonucleotides but have non-naturally occurring portions. For example, oligonucleotide analogs can contain non-naturally occurring portions, such as altered sugar moieties or inter-sugar linkages, such as a phosphorothioate oligodeoxynucleotide. Functional analogs of naturally occurring polynucleotides can bind to RNA or DNA, and include peptide nucleic acid (PNA) molecules. Such analog molecules may also bind to or interact with polypeptides or proteins.

**Oligopeptide:** An oligopeptide is defined as a linear molecule of about 50 or fewer amino acid residues.

30       **Peptide Nucleic Acid (PNA):** An oligonucleotide analog with a backbone comprised of monomers coupled by amide (peptide) bonds, such as amino acid monomers joined by peptide bonds.

35       **Probe:** A molecule that may bind to or interact with one or more polypeptides. A probe, as the term is used herein, can be any molecule that is used to challenge ("probe," "assay," "interrogate" or "screen") a polypeptide array in order to determine the binding or interaction characteristics of the arrayed polypeptides with that probe molecule. In specific embodiments of the current invention, probes may be from different and varied molecular classes. Such classes are, for instance, nucleic acids (such as single or double stranded DNA or RNA), oligo- or polypeptides (such as proteins).

protein fragments including domains or sub-domains, and mutants or variants of naturally occurring proteins), or various types of other potential polypeptide-binding molecules. Such other molecules are referred to herein generally as ligands (such as drugs, toxins, venoms, hormones, co-factors, substrates or reaction products of enzymatic reactions or analogs thereof, transition state analogs, minerals, and so forth).

Usually, a probe molecule is detectable for use in probing an array of the invention. Probes can be detectable based on their inherent characteristics (e.g., immunogenicity) or can be rendered detectable by being labeled with an independently detectable tag. The tag may be any recognizable feature that is, for example, microscopically distinguishable in shape, size, color, optical density, etc., differently absorbing or emitting of light; chemically reactive; magnetically or electronically encoded; or in some other way detectable. Specific examples of tags are fluorescent or luminescent molecules that are attached to the probe, or radioactive monomers or molecules that can be added during or after synthesis of the probe molecule. Other tags may be immunogenic sequences (such as epitope tags) or molecules of known binding pairs (such as members of the strept/avidin:biotin system). Other tags and detection systems are known to those of skill in the art, and can be used in the present invention.

Though in many embodiments of the invention a single type of probe molecule (for instance one protein) at a time will be used to assay the array, in some embodiments, mixtures of probes will be used, for instance mixtures of two proteins or two nucleic acid molecules. Such co-applied probes may be labeled with different tags, such that they can be simultaneously detected as different signals (e.g., two fluorophors that emit at different wavelengths).

**Probe standard:** A probe molecule for use as a control in analyzing an array. Positive probe standards include any probes that are known to interact with at least one of the target polypeptides of the array. Negative probe standards include any probes that are known not to interact with at least one target polypeptide of the array. Probe standards that may be used in any one system include molecules of the same class as the test probe that will be used to assay the array. For instance, if the array will be used to examine the interaction of a protein with the polypeptides of the array, the probe standard can be a protein or oligo- or polypeptide. However, this will not always be the case.

In some instances, as in certain of the kits that are subjects of this invention, a probe standard will be supplied that is unlabeled. Such unlabeled probe standards can be used in a labeling reaction as a standard for comparing labeling efficiency of the test probe that is being studied. In some embodiments, labeled probe standards will be provided in the kits.

**Probing:** As used herein, the term "probing" refers to incubating an array with a probe molecule (usually in solution) in order to determine whether the probe molecule will bind to or interact with molecules immobilized on the array. Synonyms include "interrogating," "challenging," "screening" and "assaying" an array. Thus, a universal protein array of the invention is said to be

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"probed" or "assayed" or "challenged" when it is incubated with a probe molecule (such as a polypeptide, nucleic acid molecule, or ligand).

**Protein/Polypeptide:** A biological molecule expressed by a gene or other encoding nucleic acid, and comprised of amino acids. More generally, a polypeptide is any linear chain of amino acids, usually about 50 or more amino acid residues in length.

Arrays according to the present invention include a plurality of polypeptide samples (targets) "spotted" at assignable locations on the surface of an array substrate. The polypeptide at each spot can be referred to as a target polypeptide, or target polypeptide sample. In certain embodiments, polypeptides are deposited on and bound to the array surface in a substantially native configuration, such that at least a portion of the individual polypeptides within the spot are in a native configuration. Such native configuration polypeptides are capable of binding to or interacting with molecules in solution that are applied to the surface of the array in a manner that approximates natural intra- or intermolecular interactions. Thus, binding of a molecule in solution (for instance, a probe) to a target polypeptide immobilized on an array will be indicative of the likelihood of such interactions in the natural situation (*i.e.*, within a cell).

In certain arrays of the invention, referred to as pooled arrays, at least one particular address on the array is occupied by a pooled mixture of more than one substantially pure target polypeptide. All of the addresses on the array may contain pools of polypeptide, or only some of the addresses, depending on the use of the array. For instance, in some circumstances it may be desirable to array a target polypeptide associated with one or more non-target polypeptides, for instance a stabilizing polypeptide or linker molecule. In addition, the native conformation of certain binding sites on proteins can only be assayed for probe binding when the target polypeptide is associated with other molecules, for instance when the target polypeptide natively exists as one subunit of a multimeric complex. Pooled arrays of the current invention include those on which one or more of the addresses contains a multimeric polypeptide complex. In the case of such an array, it is envisioned that different probe molecules may bind to different polypeptides within the complex of "target" polypeptides.

Although the identity of each probe in the pooled mixture at a specific address is known, the individual probes in the pool are not "separately addressable." The binding signal from a pooled address is the binding signal of the set of different (but mixed or associated) polypeptides occupying that address. In general, an address is considered to display binding of a probe molecule if at least one polypeptide occupying the address binds to the probe molecule.

Arraying pooled samples is also a powerful tool in high-throughput technologies for increasing the information that is yielded each time the array is assayed.

**Protein purification:** Polypeptides for use in the present invention can be purified by any of the means known in the art. See, *e.g.*, *Guide to Protein Purification*, ed. Deutscher, *Meth.*

*Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982.

**Purified:** The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified protein preparation is one in which the specified protein is more enriched than the protein is in its generative environment, for instance within a cell or in a biochemical reaction chamber. A preparation of substantially pure protein may be purified such that the desired protein represents at least 50% of the total protein content of the preparation. In certain embodiments, a substantially pure protein will represent at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% or more of the total protein content of the preparation.

**Recombinant:** A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques.

**Stripping:** Bound probe molecules can be stripped from an array, for instance a universal protein array, in order to use the same array for another probe interaction analysis. Any process that will remove essentially all of the first probe molecule from the array, without also significantly removing the immobilized polypeptides of the array, can be used with the current invention. By way of example only, one method for stripping a universal protein array is by washing it in stripping buffer (e.g., 1 M  $(\text{NH}_4)_2\text{SO}_4$  and 1 M urea), for instance at room temperature for about 30-60 minutes. Usually, the stripped array will be equilibrated in a low stringency wash buffer prior to incubation with another probe molecule.

**Universal Protein Array:** Universal protein arrays provide parallel analysis of the extent that a probe molecule (e.g., a detectable probe molecule) binds to or interacts with several to thousands of immobilized polypeptide molecules. Many copies of (usually) a single type of target molecule are bound to the array surface in a spot that may be, in the case of a microarray, approximately 0.1 mm or less in diameter, or will be larger in the case of a macroarray (for instance, a UPA constructed using a dot-blot or slot-blot apparatus). The target molecules immobilized on the array of a UPA are substantially pure polypeptides.

The many spots of a UPA, each containing at least two different polypeptide targets, can be arrayed in the shape of a grid, although other array configurations can be used so long as the spots of the array are addressable. The surface for arraying (the substrate) may be a glass, or other solid material, or a filter paper or other substance useful for attaching polypeptides. When interrogated with detectable probe sample (for instance, one that is labeled with a fluorescent or a radioactive tag), the binding of the probe to the array (possibly producing a pattern) indicates the relative binding affinity of the probe for each of the immobilized polypeptides. The binding of a probe to a polypeptide of the UPA can be visualized by detecting the labeled probe molecule.

In variations of the UPA technology, the detectable probe is a specific protein, polypeptide, single- or double-stranded nucleic acid, ligand or other natural or synthetic molecule, depending on the interaction(s) being tested for. Such detectable molecules are used to detect and/or quantitate interaction with the polypeptides of the UPA.

5 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In  
10 case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

## II. Universal Protein Arrays

15 Arrays of the current invention provide several advantages over prior technologies and methods used for analysis of protein-molecule interactions. Although dot blot analysis with unpurified protein preparations has been used for the detection of specific antibody-antigen interactions, use of highly purified and active recombinant or native target proteins, in an array  
20 format, to assay for interactions with a specific probe has not previously been reported. Additionally, because the UPA assay can in some embodiments be carried out under non-denaturing conditions, it provides a simple system for detecting native interactions between polypeptides and probe molecules.

Known techniques fall far short of the UPA invention disclosed herein. In the case of far-western blot analysis, protein fractions were usually analyzed by SDS-PAGE and electrotransferred  
25 to a membrane, followed by denaturation and renaturation before probing with a radiolabeled protein probe. On average, only 1-10% of the activity (without considering the loss of protein during the transfer process) could be recovered for most proteins with such a procedure (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998). In contrast, UPA analysis as described herein simply uses active proteins directly spotted onto a substrate, such as a membrane. Therefore, it is at least 10- to 100-fold more sensitive  
30 than the far-western blot assay.

Since the amount of active protein assayed for interaction with the probe on a UPA can be the amount of protein applied, the affinities of individual proteins for a specific probe molecule, either a protein or another type of biomolecule or other ligand, can be easily quantified and compared with each other.

35 Most existing assay systems were designed for a single purpose (to be probed with a single type of molecule). For example, the two hybrid system, co-immunoprecipitation, far-western blotting and GST assays were all used only for protein-protein interaction; the gel mobility shift.

footprinting and cross-linking assays were used for protein-nucleic acid (DNA or RNA) interactions, and microarrays or DNA chips were used only for nucleic acid interactions. In contrast, the same UPA as described herein has been successfully used for detection of protein-protein, protein-DNA, protein-RNA and protein-ligand interactions. It is also useful for detecting protein-metal ion interactions.

Given that the major part of the human genome sequence has been identified, that the entire genome sequence is expected to be completed by the year 2003 (Collins *et al.*, *Science*, 282, 682-689, 1998) and that most active proteins can be overexpressed in and purified from either bacteria, baculovirus or mammalian cells, the availability of 100,000 human gene products (Collins *et al.*, *Science*, 282, 682-689, 1998) will provide a rich source of proteins for UPA-mediated polypeptide interaction studies. The UPA system not only provides an alternative and efficient method to explore the mechanisms of gene expression pathways, but also a new pipeline to screen and to design new drugs, with tremendous potential for disease diagnosis.

Below are described several characteristics of the universal protein arrays of the invention. The embodiments and examples given are meant in no way to limit the invention.

#### A. Choice of polypeptide targets

The target(s) of interest will be selected according to a wide variety of methods. For example, certain targets of interest are well known and included in public databases such as GenBank or a similar commercial database. Other targets will be identified from journal articles, or from other investigations using high throughput technologies (*e.g.*, cDNA microarrays or Gene Chips), or with other techniques. In certain embodiments, the sequences of arrayed target polypeptides can be provided via an ASCII text file, for instance to assist data storage, sorting and comparison.

Any polypeptides can serve as targets for use in the subject arrays. For instance, an array could be assembled that reflects every protein encoded for by the genome of an organism. Alternatively, arrays can be designed that contain a specific family of proteins. Such families can be defined in various ways, including proteins that act in a specific cellular process (*e.g.*, transcription-related proteins), proteins that are in a linked biochemical pathway (*e.g.*, proteins involved in the respiratory pathway), proteins known to be involved in diseases, etc. Arrays can also be produced that include proteins of a specific type (*e.g.*, DNA polymerases) from various different species. Arrays of the oligopeptides or polypeptides encoded for by ESTs can also be created, and are useful for identifying the function of individual EST-linked genes and the proteins they encode.

In essence, any combination or grouping of polypeptides can be assembled together one or a set of UPAs for simultaneous analysis of interaction with one or more probes of interest.

By way of example, there are approximately 100,000 different genes in the human genome, and it is expected that all of them will be known within the next few years. With the provision of every gene in the human genome, every protein encoded for by each human gene can be arrayed on

one or a collection of UPAs, such that the entire human complement of proteins can be screened for probe interactions. Arrays can also be arranged that contain the entire collection of proteins encoded on a single human chromosome, such that a collection of 23 UPAs would encompass the entire human genome.

5 Genome-wide or chromosome-specific polypeptide arrays or array sets are not limited to the human genome. Any species for which the genome is known or becomes known could be arrayed on one or a collection of arrays according to this invention. Such non-human genomes include those from disease organisms (e.g., viruses, bacteria, parasites, etc.), research organisms (*Drosophila melanogaster*, *Caenorhabditis elegans*, *Xenopus laevis*, *Arabidopsis*, *Saccharomyces cerevisiae*,  
10 *Escherichia coli*, etc.), and so forth.

As demonstrated below (Example 3), UPA is an effective method to map protein interaction domains and DNA- or RNA-binding domains of a protein. In certain UPAs of this invention, therefore, the target polypeptides are collections of closely related sequences, for instance a series of nested polypeptide deletions of varying length or a series of polypeptides with different amino acid  
15 residues at single sites throughout the sequence. Another alternative is a collection of different domain fragments of one protein or a family of closely related proteins; the domains may be fused to another (non-target) protein. Such domain or mutation arrays can be used to determine which amino acid residues or domains are important in known or suspected binding interactions between the base target protein and the probe or probes used to assay the array.

20 Applications of the universal protein array technology are not limited to studies of transcriptional factors, although the following Examples 1-6 disclose embodiments of its use in connection with analysis of such factors. UPA analysis could also be instrumental in understanding polypeptide binding characteristics of multiple protein profiles expressed during various disease states or growth conditions, as well as in normal human or animal protein profiles, including profiles  
25 from different transgenic animals or cultured cells.

Polypeptide arrays according to this invention may also be used to perform further analysis on genes and targets discovered from, for example, high-throughput genomics, such as DNA sequencing, DNA microarrays, or SAGE (Serial Analysis of Gene Expression) (Velculescu *et al.*, *Science* 270:484-487, 1995). Polypeptide arrays according to this invention may also be used to  
30 evaluate reagents for disease or cancer diagnostics, for instance specific antibodies or probes that react with certain polypeptides from infectious organisms or from tissues at different stages of cancer development. This technology can also be used to follow progression of polypeptide changes both in the same and in different cancer types, or in diseases other than cancer. Polypeptide arrays according to this invention may be used to identify and analyze prognostic markers or markers that predict  
35 therapy outcome for various diseases or abnormal conditions, such as cancers. Arrays compiled from the proteins of hundreds of cancers derived from patients with known disease outcomes permit binding or association assays to be performed on those arrays, to determine important prognostic

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markers, or markers predicting therapy outcome, which are associated with polypeptide binding characteristics.

Polypeptide arrays according to this invention may also be used to help assess the ability of certain drugs or potential drugs to interact with target polypeptides, or the ability of such molecules to block the interaction of other probes with arrayed polypeptides.

The UPAs of this invention can be used to investigate receptor specificity of different types of known and suspected receptor molecules. Examples of receptors that can be investigated for probe-specific binding by arrays according to this invention include but are not limited to microorganism receptors (for instance, those found in fungi, protozoa, and bacteria, especially bacterial strains that are resistant to antibiotics); hormone receptors (including those involved in diabetes, growth regulation, vasoregulation, and so forth); and opiate receptors (involved in biological responses, for instance to addictive drugs).

Also envisioned are arrays that are custom produced for the researcher, with an arrayed collection of polypeptides tailored to a specific research project, research system, etc.

Not in any way intending to be limited to the list below, the following is a list of the types of collections of polypeptides that can be arrayed on a UPA according to this invention: all or substantially all the proteins encoded for by the genome of an organism; all or substantially all the proteins encoded for by a chromosome of an organism; proteins expressed in a cell during a particular growth phase or environmental condition; proteins expressed in a cell under a particular abnormal state (such as cancer, disease, or infection); proteins expressed in cells at various times during the progression of a disease or condition (e.g., during progression of a tumor, or development of a chronic disease such as Alheizmers); proteins expressed in a particular cell type; proteins from a particular protein family (e.g., DNA polymerases, cell surface proteins, transmembrane proteins or fragments [such as soluble fragments] thereof, oncogene proteins, tumor suppressor proteins, and so forth); proteins that show sequence homology to each other; proteins that share secondary structural characteristics; proteins that associate to form multimeric complexes (e.g., the subunits of a ribosome or a membrane ATPase); viral epitopes; domains of proteins; proteins from different species; and collections of fragments of any of these protein collections.

#### B. Production of substantially pure target polypeptides

Polypeptides for use as targets on the subject arrays can be produced by any technique that yields native protein. These techniques in general include expression from engineered DNA constructs, extraction from native samples (e.g., clinical samples), or de novo synthesis of oligopeptide or polypeptide fragments.

Expression of the target polypeptides can be carried out using well known techniques. For instance, partial or full-length cDNA sequences, which encode the protein of interest as a target on the UPA, may be ligated into bacterial expression vectors. Methods for expressing large amounts of protein from a cloned gene introduced into *Escherichia coli* (*E. coli*) may be utilized for the



production and purification of intact, native target proteins. Methods and plasmid vectors for producing fusion proteins and intact native proteins in bacteria are described in Sambrook *et al.* (Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, Ch. 17, CSHL, New York, 1989). Such fusion proteins may be made in large amounts and are easy to purify. Native proteins can be produced in bacteria by placing a strong, regulated promoter and an efficient ribosome-binding site upstream of the cloned gene. If low levels of protein are produced, additional steps may be taken to increase protein production; if high levels of protein are produced, purification is relatively easy. Suitable methods are presented in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989) and are well known in the art. Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods for extracting proteins from these aggregates are described by Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, Ch. 17, CSHL, New York, 1989). Vector systems suitable for the expression of *lacZ* fusion genes include the pUR series of vectors (Ruther and Muller-Hill, *EMBO J.* 2:1791, 1983), pEX1-3 (Stanley and Luzio, *EMBO J.* 3:1429, 1984) and pMR100 (Gray *et al.*, *Proc. Natl. Acad. Sci. USA* 79:6598, 1982). Vectors suitable for the production of intact native proteins include pKC30 (Shimatake and Rosenberg, *Nature* 292:128, 1981), pKK177-3 (Amann and Brosius, *Gene* 40:183, 1985) and pET-3 (Studiar and Moffatt, *J. Mol. Biol.* 189:113, 1986).

#### C. Choice of array format and structure

UPAs may vary significantly in their structure, composition, and intended functionality. The UPA system is amenable to use in either a macroarray or a microarray format, or a combination thereof. Such arrays can include, for example, at least 50, 100, 150, 200, 500, 1000, or 5000 or more array elements (such as spots). In the case of macro-UPAs, no additional sophisticated equipment is usually required to detect the bound probe on the UPA, though quantification may be assisted by known automated scanning and/or quantification techniques and equipment. Thus, macro-UPA analysis can be carried out in most research laboratories and biotechnology companies, without the need for investment in specialized and expensive reading equipment.

Examples of substrates for UPAs include glass (*e.g.*, functionalized glass), Si, Ge, GaAs, GaP, SiO<sub>2</sub>, SiN<sub>4</sub>, modified silicon nitrocellulose, polyvinylidene fluoride, polystyrene, polytetrafluoroethylene, polycarbonate, nylon, fiber, or combinations thereof. Array substrates can be stiff and relatively inflexible (*e.g.*, glass or a supported membrane) or flexible (such as a polymer membrane). One commercially available microarray system that can be used with the arrays of this invention is the FAST™ slides system (Schleicher & Schuell, Dassel, Germany), which incorporates a patch of polymer on the surface of a glass slide.

In general, a target on the array should be discrete, in that signals from that target can be distinguished from signals of neighboring targets, either by the naked eye (macroarrays) or by scanning or reading by a piece of equipment or with the assistance of a microscope (microarrays).

Macro-UPAs are often arrayed on polymer membranes, either supported or not, and can be

of any size, but typically will be greater than a square centimeter. Other examples of macroarray substrates include glass, fiber, plastic and metal. Macroarrays are generally used when the number of polypeptides in the target set is relatively small, on the order of tens to hundreds of samples, however macroarrays with a larger number of array elements can be used on large substrates. Spot arrangement on the macroarray is such that individual spots can be distinguished from each other when the sample is read; typically, the diameter of the spot is about equal to the spacing between individual dots.

Sample spots on macroarrays are of a size large enough to permit their detection without the assistance of a microscope or other sophisticated enlargement equipment. Thus, spots may be as small as about 0.1 mm across, with a separation of about the same distance, and can be larger. Larger sample spots on macroarrays, for example, may be about 0.5, 1, 2, 3, 5, 7, or 10 mm across. Even larger spots may be larger than 10 mm (1 cm) across, in certain specific embodiments. The array size will in general be correlated the size of the sample spots applied to the array, in that larger spots will usually be found on larger arrays, while smaller spots may be found on smaller arrays. This correlation is not necessary to the invention, though.

In microarray UPAs, a common feature is the small size of the target array, for example on the order of a squared centimeter or less. A squared centimeter (1 cm by 1 cm) is large enough to contain over 2,500 individual target spots, if each spot has a diameter of 0.1 mm and spots are separated by 0.1 mm from each other. A two-fold reduction in spot diameter and separation can allow for 10,000 such spots in the same array, and an additional halving of these dimensions would allow for 40,000 spots. Using microfabrication technologies, such as photolithography, pioneered by the computer industry, spot sizes of less than 0.01 mm are feasible, potentially providing for over a quarter of a million different target sites. The power of microarray-format UPAs resides not only in the number of different polypeptides that can be probed simultaneously, but also in how little protein is need for the target.

The amount of polypeptide target sample that is applied to each address of an array will be largely dependent on the array format used. For instance, microarrays will generally have less polypeptide applied at each address than will macroarrays. By way of example, individual targets on a macroarray can be applied in the amount of about 1 pmol or greater, for instance about 3 pmol, about 5 pmol, about 7.5 pmol, about 10 pmol, about 15 pmol or more. In contrast, samples applied to individual spots on a microarray will usually be less than 1 pmol in each spot, for instance, about .8 pmol, about 0.5 pmol, about 0.3 pmol, about 0.1 pmol, about .05 pmol or less.

In addition, the surface area of sample application for each "spot" will influence how much polypeptide is immobilized on the array surface. Thus, a larger spot (having a greater surface area) will generally accept or require a greater amount of target molecule than a smaller sample spot (having a smaller surface area).

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The target polypeptide itself (e.g., the length of the polypeptide, its primary and secondary structure, its binding characteristics in relation to the array substrate, etc.) will influence how much of each target polypeptide is applied to an array. Optimal amounts of target molecule for application to an array of the invention can be easily determined, for instance by applying varying amounts of the target polypeptide to an array surface and probing the array with a probe molecule known to interact with that target. In this manner, it is possible for one of ordinary skill in the art to empirically determine of range of target molecule amounts that produce interpretable results.

Another way to describe an array is its density – the number of samples in a certain specified surface area. For macroarrays of the current invention, array density will usually be between about one target per squared decimeter (or one target address in a 10 cm by 10 cm region of the array substrate) to about 50 targets per squared centimeter (50 targets within a 1 cm by 1 cm region of the substrate). For microarrays, array density will usually be one target per squared centimeter or more, for instance about 50, about 100, about 200, about 300, about 400, about 500, about 1000, about 1500, about 2,500, about 5,000, about 10,000, about 50,000, about 100,000 or more targets per squared centimeter.

#### D. Application of targets to arrays

Targets on the array may be made of oligopeptides, polypeptides, proteins, or fragments of these molecules. Oligopeptides, containing between about 8 and about 50 linked amino acids, can be synthesized readily by chemical methods. Photolithographic techniques allow the synthesis of hundreds of thousands of different types of oligopeptides to be separated into individual spots on a single chip, in a process referred to as *in situ* synthesis, as has been done with oligonucleotide arrays.

Longer polypeptides or proteins, on the other hand, contain up to several thousand amino acid residues, and are not as easily synthesized through *in vitro* chemical methods. Instead, polypeptides and proteins for use in UPAs are usually expressed using one of several well known cellular expression systems, including those described above. Alternatively, proteins can be isolated from their native environment, for instance from tissue samples or environmental samples, or from expression chambers in the case of engineered expressed polypeptides. After extraction and appropriate purification, the polypeptide can be deposited onto the array using any of a variety of techniques.

In the methods disclosed in this applications, target polypeptides can be delivered to the substrate of the array by various different mechanisms. One is by flowing within a channel defined on predefined regions of the array substrate. Typical "flow channel" application methods for applying the polypeptides to arrays of the present invention are represented by dot-blot or slot-blot systems (see, e.g., U.S. Patents No. 4,427,415 and 5,283,039). One alternative method for applying the targets to the array substrate is "spotting" the target polypeptide on predefined regions (each corresponding to an array address). In a spotting technique, the target molecules are delivered by directly depositing (rather than flowing) relatively small quantities of them in selected regions. For

instance, a dispenser can move from address to address, depositing only as much target as necessary at each stop. Typical dispensers include an ink-jet printer or a micropipette to deliver the target in solution to the substrate and a robotic system to control the position of the micropipette with respect to the substrate. In other embodiments, the dispenser includes a series of tubes, a manifold, an array of pipettes, or the like so that the target polypeptides can be delivered to the reaction regions simultaneously.

Usually, the target polypeptides are deposited on the array substrate in such a way that they are substantially irreversibly bound to the array. For example, a target may be bound such that no more than 30% of the polypeptide on the array at the end of the binding process can be washed off using buffers of the UPA system (e.g., low or high salt buffers or stripping buffers). In other embodiments, no more than 25%, no more than 20%, no more than 15%, no more than 10%, no more than 5%, or no more than 3% of the polypeptide on the array at the end of the binding process can be washed off using buffers of the UPA system.

Depending on the array substrate used, the substrate alone may substantially irreversibly bind the target without further linking being necessary (e.g., nitrocellulose and PVDF membranes). In other instances, a linking or binding process must be performed to ensure binding of the polypeptides. Examples of linking processes are known to those of skill in the art, as are the substrates that require such a linking process in order to bind polypeptide molecules. The target polypeptides optionally may be attached to the array substrate through linker molecules.

In certain embodiments, the non-sample regions of the array surface (those regions of the array surface that do not contain target molecules) are blocked in order to prevent or inhibit binding of the probe molecules directly to the array surface.

It is beneficial in certain embodiments to apply a known amount of each target polypeptide on the array. In particular embodiments, an essentially equal amount of each target polypeptide is applied to each spot. Quantification and equivalent application of the targets permits comparison of probe binding affinity between the different targets. Measurements of the amount of specific target proteins may be carried out through many techniques well known in the art. These include quantitative immunoblot analysis, enzyme activity assays (where appropriate), and commercially available protein quantification kits (e.g., Bio-Rad protein assay systems), which determine the concentration of protein in a sample regardless of biological characteristics of the specific protein being measured.

Many other techniques could be used to measure the amount of a target protein present in a sample. For instance, the amount of target protein in a sample could be measured using a quantitative enzyme-linked immunosorbant assay ('ELISA') as described by Aboagye-Mathiesen *et al.* (*Placenta* 18:155-61, 1997).

In certain arrays of the invention, referred to as pooled arrays, at least one particular address on the array is occupied by a pooled mixture of more than one substantially pure target polypeptide.

All of the addresses on the array may contain pools of polypeptide, or only some of the addresses, depending on the use of the array. For instance, in some circumstances it may be desirable to array a target polypeptide associated with one or more non-target polypeptides, for instance a stabilizing polypeptide or linker molecule. In addition, the native conformation of certain binding sites on proteins can only be assayed for probe binding when the target polypeptide is associated with other molecules, for instance when the target polypeptide natively exists as one subunit of a multimeric complex. Pooled arrays of the current invention include those on which one or more of the addresses contains a multimeric polypeptide complex. In the case of such an array, it is envisioned that different probe molecules may bind to different polypeptides within the complex of "target" polypeptides.

Although the identity of each probe in the pooled mixture at a specific address is known, the individual probes in the pool are not "separately addressable." The binding signal from a pooled address is the binding signal of the set of different (but mixed or associated) polypeptides occupying that address. In general, an address is considered to display binding of a probe molecule if at least one polypeptide occupying the address binds to the probe molecule.

Arrayed pooled samples is also a powerful tool in high-throughput technologies for increasing the information that is yielded each time the array is assayed. Methods for analyzing signals from arrays containing pooled samples have been described, for instance in U.S. Patent No. 5,744,305, incorporated herein by reference in its entirety.

#### E. Choice of probe molecule(s)

Any molecule that might bind to or interact with one or more polypeptides can be used as a probe with the disclosed arrays. In specific embodiments of the current invention, probes may be from different molecular classes (e.g., nucleic acids, oligo- or polypeptides, or various types of ligands). Probes (especially those that are polymeric chains) may be of various lengths, and different results may be obtained from the same array by using related probe molecules of different length. Likewise, varying the sequence of polymeric chain probes may provide valuable binding data.

Though in many embodiments of the invention a single type of probe molecule (for instance one protein) at a time will be used to assay the array, in some embodiments, mixtures of probes will be used simultaneously, for instance mixtures of two proteins or two nucleic acid molecules. Simultaneous multiple-probing (e.g. double-probing) can be used to detect either competitive binding or binding systems that require the interaction of more molecules than just one polypeptide target and one probe molecule.

#### F. Labeling and detection of probe molecule(s)

Usually, probe molecules used to assay the disclosed UPAs are detectable. Probes can be detectable based on their inherent characteristics (e.g., immunogenicity) or can be rendered detectable by being labeled with an independently detectable tag. Such tags include fluorescent or luminescent molecules that are attached to the probe, or radioactive monomers or molecules that can

be added during or after synthesis of the probe molecule. Other tags may be immunogenic sequences (such as epitope tags) or molecules of known binding pairs (such as members of the strept/avidin:biotin system). Other tags and detection systems are known to those of skill in the art, and can be used in the present invention.

5 Labeling different probes with different tags to enable simultaneous detection of binding of two or more probes on the polypeptides of an array. Multiple-label challenges to an array of this invention can also be used to examine any competitive binding between the two arrays on different polypeptides of the array. For competitive binding assays, however, only one of the probes needs to be detectable.

10 G. Computer assisted (automated) detection and analysis of UPAs

The data generated by assaying a universal protein array according to this invention can be analyzed using known computerized systems. For instance, the array can be read by a computerized "reader" or scanner and the quantification of the binding of probe to individual addresses on the array carried out using computer algorithms. Such analysis of the array can be referred to as "automated  
15 detection" in that the data is being gathered by an automated reader system.

In the case of labels that emit detectable electromagnetic wave or particles, the emitted light (e.g., fluorescence or luminescence) or radioactivity can be detected by very sensitive cameras, confocal scanners, image analysis devices, radioactive film or a Phosphorimager, which capture the signals (such as a color image) from the array. A computer with image analysis software detects this  
20 image, and analyzes the intensity of the signal for each probe location in the array. Signals can be compared between spots on a single array, or between arrays (such as a single array that is sequentially probed with multiple different probe molecules).

Computer algorithms can also be used for comparison between spots on a single array or on multiple arrays. In addition, the data from an array can be stored in a computer readable form.

25 Certain examples of automated array readers (scanners) will be controlled by a computer and software programmed to direct the individual components of the reader (e.g., mechanical components such as motors, analysis components such as signal interpretation and background subtraction). Optionally software may also be provided reader to control a graphic user interface and one or more systems for sorting, categorizing, storing, analyzing, or otherwise processing the data  
30 output of the reader.

To "read" an array according to this invention, an array that has been assayed with a detectable probe to produce binding (e.g., a binding pattern) can be placed into (or onto, or below, etc., depending on the location of the detector system) the reader and a detectable signal indicative of probe binding detected by the reader. Those addresses at which the probe has bound to immobilized  
35 polypeptide sample provide a detectable signal, e.g., in the form of electromagnetic radiation. These detectable signals could be associated with an address identifier signal, identifying the site of the complex. The reader gathers information from each of the addresses, associates it with the address

identifier signal, and recognizes addresses with a detectable signal as distinct from those not producing such a signal. The reader is also capable of detecting intermediate levels of signal, between no signal at all and a high signal, such that quantification of signals at individual addresses is enabled.

5 Certain readers that can be used to collect data from the arrays of this invention, especially those that have been probed using a fluorescently tagged molecule, will include a light source for optical radiation emission. The wavelength of the excitation light will usually be in the UV or visible range, but in some situations may be extended into the infra-red range. A beam splitter can direct the reader-emitted excitation beam into the object lens, which for instance may be mounted such that it  
10 can move in the x, y and z directions in relation to the surface of the array substrate. The objective lens focuses the excitation light onto the array, and more particularly onto the (polypeptide) targets on the array. Light at longer wavelengths than the excitation light is emitted from addresses on the array that contain fluorescently-labeled probe molecules (*i.e.*, those addresses containing a polypeptide to which the probe binds).

15 In certain embodiments of the invention, the array may be movably disposed within the reader as it is being read, such that the array itself moves (for instance, rotates) while the reader detects information from each address. Alternatively, the array may be stationary within the reader while the reader detection system moves across or above or around the array to detect information from the addresses of the array. Specific movable-format array readers are known and described, for  
20 instance in U.S. Patent No. 5, 922,617, hereby incorporated in its entirety by reference. Examples of methods for generating optical data storage focusing and tracking signals are also known (see, for example, U.S. Pat. No. 5,461,599, hereby incorporated in its entirety by reference).

For the electronics and computer control, a detector (*e.g.*, a photomultiplier tube, avalanche detector, Si diode, or other detector having a high quantum efficiency and low noise) converts the  
25 optical radiation into an electronic signal. An op-amp first amplifies the detected signal and then an analog-to-digital converter digitizes the signal into binary numbers, which are then collected by a computer.

### 30 III. Examples

#### Example 1: Preparation of a UPA

##### Methods and Materials

35 To identify target proteins to which the transcriptional coactivator p52 will bind, the protein array system for which a target arrangement key is shown in Table 1 was provided. The general transcription factors, activators and coactivators arrayed were overexpressed either in bacteria, baculovirus or in mammalian cells and purified to near homogeneity as previously described (Chiang

*et al.*, *EMBO J.*, 12, 2749-2762, 1993; Kershner *et al.*, *J. Biol. Chem.*, 18, 34444-34453, 1998; Luo *et al.*, *Cell*, 71, 231-241, 1992; Jackson and Tjian, *Proc. Natl. Acad. Sci. USA*, 86, 1781-1785, 1989; Ge *et al.*, *Methods Enzymol.*, 274, 57-71, 1996). The serine-arginine (SR) protein fraction was prepared from HeLa cell nuclear extracts essentially according to Zahler *et al.* (*Genes Dev.*, 6, 837-847, 1992). GST-nucleolin fusion protein (GST-Nu, address 12e/f) was prepared by overexpressing plasmid GST-HNB (provided by Dr M. Srivastava), which contains nucleolin coding sequence positions 290-707, in bacteria and purified on a glutathione-Sepharose column. Glutathione S-transferase fused to a HMK site (RRASV) (GST-K) (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998) was used as a negative control in the experiments.

10 An average of 7.5 pmol (normalized by Bio-Rad protein assay, Bio-Rad, Hercules, CA) of each of the 48 highly purified proteins (or fractions) was spotted on a 12 x 8 cm nitrocellulose membrane using a 96-well dot blot apparatus (Bio-Rad, Hercules, CA). This apparatus provides sample application to a membrane to form an array arranged in twelve rows and eight columns. The arrangement of the polypeptide targets in the array is shown in Table 1, which corresponds to the  
15 array results shown in Figures 1 and 2. Each sample was duplicated in two adjacent wells to provide a useful internal control.

Each sample was diluted to 100 µl with buffer A100 (100 mM KCl, 10% glycerol, 20 mM HEPES Na pH 7.9, 0.2 mM EDTA, 10 mM 2-mercaptoethanol and 0.5 mM PMSF) and duplicated in two adjacent wells. Each well was rinsed with 2 x 500 µl buffer A100 and the vacuum kept for 3-5  
20 minutes. After removal from the dot blot apparatus, the protein array was rinsed with two changes of buffer A100.



Table 1\*

	a	b	c	d	e	f	g	h
1	(1) TFIIA	TFIIA	(2) TFIIB	TFIIB	(3) TBP	TBP	(4) f:TFIID	f:TFIID
2	(5) TFIIE	TFIIE	(6) TFIIF	TFIIF	(7) f:TFIIH	f:TFIIH	(8) Pol II	Pol II
3	(9) RXR	RXR	(10) TR	TR	(11) Oct 1	Oct 1	(12) Sp1	Sp1
4	(13) G4-94	G4-94	(14) G4-147	G4-147	(15) G4-AH	G4-AH	(16) G4-VP16	G4-VP16
5	(17) G4-CTF	G4-CTF	(18) G4-Sp1	G4-Sp1	(19) G4-E1A	G4-E1A	(20) G4-IE	G4-IE
6	(21) G4-Tat	G4-Tat	(22) PC4-P	PC4-P	(23) PC4-N	PC4-N	(24) PC4-C	PC4-C
7	(25) PC4-ΔS	PC4-ΔS	(26) PC4-m1	PC4-m1	(27) PC4-m2	PC4-m2	(28) PC4-m3	PC4-m3
8	(29) PC4-m4	PC4-m4	(30) PC4-m5	PC4-m5	(31) PC4-m6	PC4-m6	(32) PC4-m7	PC4-m7
9	(33) PC4-wt	PC4-wt	(34) p52	p52	(35) p75	p75	(36) p75-C	p75-C
10	(37) p300-C	p300-C	(38) PCAF	PCAF	(39) PCAF-C	PCAF-C	(40) TAF250	TAF250
11	(41) Topo I (wt)	Topo I (wt)	(42) Topo I (mt)	Topo I (mt)	(43) Topo I (wt)*	Topo I (wt)*	(44) Topo I (nati)	Topo I (nati)
12	(45) ASF	ASF	(46) SR (+nucl)	SR (+nucl)	(47) GST-Nu	GST-Nu	(48) GST-K	GST-K

\* Abbreviations used in Table 1 are explained above, in the Abbreviations section (1A).

5 Each sample was duplicated in two adjacent wells. The actual size of the membrane is 12 X 8 cm (height X width) with eight columns and twelve rows.

#### Example 2: Removal of probe molecules from the UPA.

10

#### Methods and Materials

The same universal protein array that was prepared in Example 1 was reused with a protein probe (Example 3), a dsDNA probe (Example 4), a ssDNA probe (Example 4), a RNA probe (Example 5) and a ligand probe (Example 6). After each use, the filter was stripped with buffer A containing 1 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 1 M urea at room temperature for 30-60 minutes. Then the stripped array was equilibrated with buffer A100 before being incubated with another probe.

15

Example 3: Interaction with a protein probe.

Methods and Materials

Purified GST-K-p52 protein (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998) was labeled by heart muscle kinase (HMK) in a 50  $\mu$ l reaction containing 10  $\mu$ g of substrate protein, 40  $\mu$ Ci [ $\gamma$ - $^{32}$ P]ATP and 10 U of the catalytic subunit of Ca-independent protein kinase A from bovine heart (Sigma, St. Louis, MO) at 30°C for 30 minutes. The  $^{32}$ P-labeled protein was purified through glutathione-Sepharose beads to separate uncoupled free nucleotide. In the case of the ASF/SF2 probe, pET11a-6H(K)-ASF/SF2 was created by inserting the ASF/SF2 coding region into the vector pET11a-6H(K) (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998) and overexpressed in *Escherichia coli* cells. Recombinant protein was affinity purified and labeled by HMK *in vitro* as described above. Pre-treatment took place in buffer A100 containing 1% non-fat milk at room temperature for at least 30 minutes. The array was then incubated with 30-50 ng probe/ml buffer A100 (+1% milk) at 4°C for over 12 hours. After incubation, the array was sequentially washed with three changes of buffer A100 (100 mM KCl), A500 (500 mM KCl) and A1000 (1000 mM KCl). The resulting signals were visualized by autoradiography (exposure from 30 minutes to 10 hours) and quantified with a densitometer (Molecular Dynamics, Sunnyvale, CA).

Results

GST-K-p52 was labeled *in vitro* with [ $\gamma$ - $^{32}$ P]ATP by HMK (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998) and further purified through glutathione-Sepharose beads. The protein array was first treated with buffer A100 containing 1% non-fat milk and then incubated with  $^{32}$ P-labeled GST-K-p52 as described in Materials and Methods. The filter was extensively washed with buffer A containing 100, 500 and 1000 mM KCl prior to each autoradiographic analysis. A low salt wash (with 100 mM KCl) allowed the detection of most possible interactions (Fig. 1A), while a high salt (with 500-1000 mM KCl) allowed the detection of highly specific and high affinity interactions (Fig. 1B). No significant difference was found between the 500 and 1000 mM salt washes. The relative affinity of each tested protein for the probe could be measured with either a densitometer or a phosphorimager (Fig. 1C). Among all 48 proteins (or fractions), the SR protein fraction (addresses 12c/d) and the recombinant GST-nucleolin (addresses 12e/f) had the highest affinities for the transcriptional coactivator p52.

It has previously been shown that, in addition to the ability to interact specifically with a 34 kDa doublet corresponding to the splicing factor ASF/SF2, p52 could also interact strongly with a 100 kDa protein found to be present in the SR fraction by far-western blot analysis (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998). Protein microsequence analysis indicated that the 100 kDa band isolated from the SR protein fraction contained two proteins, nucleolin and DNA topoisomerase I (topo I). In the present experiment, p52 strongly interacted with the recombinant GST-nucleolin but not with

topo I, either recombinant proteins expressed in baculovirus (Fig. 1, addresses 11a-f) or naturally purified protein from mammalian cells (addresses 11g/h). This observation demonstrates that p52 interacts with the nucleolin rather than the topo I present in the SR protein fraction, which is consistent with the recent observation that nucleolin is a component of the multiprotein complex associated with p52 in HeLa cells.

Nucleolin has been implicated in regulating pre-rRNA processing (Bouvet *et al.*, *EMBO J.*, 16, 5235-5246, 1997), pre-mRNA splicing (Ishikawa *et al.*, *Mol. Cell. Biol.*, 13, 4301-4310, 1993), B cell-specific transcription (Hanakahi *et al.*, *Proc. Natl. Acad. Sci. USA*, 94, 3605-3610, 1997), unwinding DNA, RNA or DNA-RNA duplexes (Tuteja *et al.*, *Gene*, 28, 143-148, 1995) and mediating cell doubling time in human cancer cells (Derenzini *et al.*, *Lab. Invest.*, 73, 497-502, 1995). Like the splicing factor ASF/SF2, nucleolin also contains RNP type RNA-binding domains as well as RGG repeats (Bouvet *et al.*, *EMBO J.*, 16, 5235-5246, 1997; Valdez *et al.*, *Mol. Immunol.*, 32, 1207-1213, 1995). Its activity can be modulated through mitosis-specific phosphorylation by p34cdc2 kinase or casein kinase II (Tuteja *et al.*, *Gene*, 28, 143-148, 1995). Therefore, it would be interesting to further examine the biological significance of nucleolin interaction with the general transcriptional coactivator and splicing regulator p52.

In addition to GST-K-p52, other protein probes have also been tested in the UPA system. Figure 3 shows the binding activity of <sup>32</sup>P-labeled splicing factor ASF/SF2, a member of the SR protein family, to 16 different selected proteins in a 4 by 4 array (Fig. 3A). ASF/SF2 significantly bound to five of the 16 proteins, including the affinity-purified TFIID complex (Fig. 3B and C, address 2d), retinoid-X receptor (address 3a), histone H1 (address 3c), co-histones (address 3d) and ASF/SF2 itself (address 4b). However, after washing the UPA with 500 mM KCl, ASF/SF2 appeared to have the highest affinity for itself (Fig. 3C, address 4b), which is in agreement with the previous observation that *in vitro* translated ASF/SF2 could strongly bind to GST-ASF/SF2 in a GST pull down assay (Xiao and Manley, *EMBO J.*, 17, 6359-6367, 1998). However, ASF/SF2 also showed high affinity for the TFIID complex. Since ASF/SF2 did not interact with TBP (address 2c), ASF/SF2 might interact directly with TBP-associated factors. Whether such an interaction reflects the function of TFIID or ASF/SF2 in transcription or pre-mRNA splicing or coupling of these could also be investigated using the disclosed UPA technology. Taken together, these experiments demonstrate that UPA can be used to detect protein interactions with various targets.

Using the same UPA, it is shown that PC4 with a single point mutation (Phe→Pro) at position 77 lost both dsDNA- and ssDNA-binding activity (Fig. 2A and B, addresses 8c/d), but still retained RNA-binding activity (Fig. 2C, addresses 8c/d). In contrast, phosphorylation of PC4 by casein kinase II stimulated the DNA-binding activity (Fig. 2A and B, addresses 6c/d), but reduced its RNA-binding activity (Fig. 2C, addresses 6c/d). These observations demonstrate that UPA is an effective method to map protein interaction domains and DNA- or RNA-binding domains of a protein.

**Example 4: Interaction with a DNA probe.****Methods and Materials**

5 To test whether the UPA system could also be used to detect interactions with other (e.g., biological) molecules, the same array was stripped (see Example 2) and reprobed with a <sup>32</sup>P-labeled double-stranded oligonucleotide (64 bp) containing the adenovirus major late core promoter elements.

10 A double-stranded (ds) oligonucleotide (64 bp with plus strand 5'-GGGGGGCTATAAAA-  
GGGGGTGGGGGCGCGTTCGTCCTCACTCTCTCCGCATCGCTGTCTGCG and minus strand  
5'-CCCTCGCAGACAGCGATGCGGAAGAGAGTGAGGACGAACGCGCCCCACCCCTTTT-  
ATAGCCC) corresponding to the adenovirus major late promoter region from -39 to +29 was  
labeled at the 3'-end of the minus strand with Klenow fragment in the presence of [<sup>32</sup>P]dCTP. After  
15 labeling, the free nucleotides were separated from the probe by passing the labeling reaction through  
a G-50 nick column (Pharmacia Biotech, United Kingdom). Pre-treatment took place with buffer A  
containing 60 mM KCl, 2x Denhardt's solution and 25 µg/ml poly(dG·dC) (Sigma, St. Louis, MO) at  
room temperature for 30 minutes. For interaction, 5 ng/ml of <sup>32</sup>P-labeled double-stranded (ds)DNA  
was added to the same buffer and incubation was carried out at 4° C for >12 hours. The array was  
then sequentially washed with three changes of buffer A100, A500 and A1000 followed by  
20 autoradiography and quantification.

To analyze the array with a single-stranded (ss)DNA probe, the 64-mer minus strand of the  
dsDNA probe was labeled at the 5'-end by T4 polynucleotide kinase in the presence of γ-[<sup>32</sup>P]ATP.  
Other conditions were exactly the same as those for the dsDNA probe.

**Results**

25 The results shown in Figure 2A indicate that, after washing with 500 mM salt,  
phosphorylated PC4 (PC4-P, addresses 6c/d), an inactive form of a previously described  
transcriptional coactivator (Ge *et al.*, *Proc. Natl. Acad. Sci. USA*, 91, 12691-12695, 1994), purified  
from HeLa cells had the highest affinity for the tested dsDNA probe among 48 samples (see  
30 quantification in Table 2). PC4-P had 3- to 5-fold higher affinity for dsDNA compared to other PC4  
derivatives, including wild-type PC4 (addresses 9a/b). In contrast, a single amino acid change at  
position 77 (Phe→Pro) completely abolished the dsDNA binding ability of PC4 (addresses 8c/d).  
These results are in agreement with the observations reported recently using gel mobility shift assays  
that phosphorylated PC4 bound bubble DNA with higher affinity and the region around position 77  
35 was critical for the DNA-binding activity of PC4 (Werten, *et al.*, *EMBO J.*, 17, 5103-5111, 1998).

Although it is known that TBP can specifically bind the present probe, the signal is relatively weak compared to other DNA-binding proteins. This result is consistent with the observation from gel mobility shift assays that the binding activity of TBP to TATA box-containing DNA was barely detectable. However, it can be significantly enhanced by the presence of another transcription factor, TFIIA (Orphanides *et al.*, *Genes Dev.*, 10, 2657-2683, 1996). On the other hand, however, many other general (non-sequence-specific) DNA-binding proteins had much stronger signals than TBP, suggesting that the present system may not be suitable for determining the binding activity of sequence-specific DNA-binding (and/or RNA-binding) proteins. ASF/SF2 was identified as an RNA-binding protein playing an essential role(s) in pre-mRNA splicing. Both the recombinant ASF/SF2 (addresses 12a/b) and the native ASF/SF2-containing SR protein fraction (12c/d) bound dsDNA as well as ssDNA (see below) very strongly, even tighter than most of the DNA-binding proteins tested (see quantification in Table 2), indicating that ASF/SF2 is also a DNA-binding protein. After the array was analyzed with a ssDNA probe (Fig. 2B), although several differences were observed, the overall pattern of protein-ssDNA interactions was similar to that of protein-dsDNA interactions, suggesting that most DNA-binding proteins are capable of binding both dsDNA and ssDNA.

**Example 5: Interaction with a RNA probe.**

**20    Methods and Materials**

An SV40 early pre-mRNA was synthesized *in vitro* from the plasmid pSVi66 by SP6 RNA polymerase as previously described (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998). Interaction was carried out at 4° C for >12 hours in the presence of 20 mM HEPES Na pH 7.9, 5% glycerol, 10 mM 2-mercaptoethanol, 0.2 mM EDTA Na pH 8.0, 60 mM KCl, 2 mM MgCl<sub>2</sub>, 0.5 mg/ml BSA, 25 µg/ml tRNA and ~5 ng/ml <sup>32</sup>P-labeled SV40 early pre-mRNA. The array was then sequentially washed and visualized by autoradiography as described for the DNA probes.

**Results**

This protein array system was also used successfully to analyze interactions with an RNA probe transcribed from the SV40 early region-containing plasmid pSVi66 (Ge *et al.*, *Mol. Cell*, 2, 751-759, 1998). Several interesting observations were revealed (see Fig. 2C). First, phosphorylation by casein kinase II *in vivo* apparently decreased the affinity of PC4 for the RNA probe (addresses 6c/d in Fig. 2C; see also Table 2), although it increased the affinity of PC4 for both the dsDNA and ssDNA probes (addresses 6c/d in Fig. 2A and B). Second, in contrast to the DNA-binding activity, the RNA-binding activity of PC4 was not significantly affected by the mutation at position 77 (addresses 8c/d in Fig. 2C). Third, both p52 and p75 strongly bind the RNA probe (addresses 9c-h in Fig. 2C), but did not significantly bind either the dsDNA or ssDNA probe in this assay (addresses 9c-

h in Fig2A and B). Finally, PCAF, a p300/CBP-associated factor that functions as a histone (Ogryzko *et al.*, *Cell*, 87, 953-959, 1996), could bind the RNA probe very strongly (addresses 10c-f in Fig. 2C; see Table 2 for quantification), suggesting a possible role of PCAF in RNA metabolism.

#### 5 Example 6: Interaction with a ligand probe

##### Methods and Materials

L-3,5,3'-[<sup>125</sup>I]Triiodothyronine (T3) was purchased from NEN (Boston, MA, catalog no. NEX110H). The interaction conditions were essentially the same as for the RNA probe except that  
10 tRNA was omitted and 0.3 µCi/ml [<sup>125</sup>I]T3 was added instead of the RNA probe.

##### Results

This protein array system was also used successfully to analyze interactions with a [<sup>125</sup>I]-labeled ligand, T3. Only the recombinant thyroid hormone receptor bound [<sup>125</sup>I]-labeled T3 strongly  
15 and specifically (addresses 3c/d in Fig. 2D).

Table 2

#	Position	Protein/source	p52 *	ds DNA *	ss DNA *	RNA *	Function/Description
1	1a/b	TFIIA/bacteria *	12.3	0.9	15.1	0.3	class II gene transcription
2	1c/d	TFIIB/bacteria *	0	0	0.2	0	class II gene transcription
3	1e/f	TBP/bacteria *	5.1	0.5	5.9	35.8	class II gene transcription
4	1g/h	f:TFIID/HeLa *	8	3.3	1.9	12.4	class II gene transcription
5	2a/b	TFIIE/bacteria *	0	0	0.2	0.6	class II gene transcription
6	2c/d	TFIIF/bacteria *	0.7	0	0.3	1.4	class II gene transcription
7	2e/f	f:TFIIH/HeLa *	1.4	2.5	0.4	4.7	class II gene transcription
8	2g/h	RNA pol II/HeLa *	9	22.4	10.8	6.1	class II gene transcription
9	3a/b	RXR/bacteria	30	11.2	37.9	34.6	activator (retinoid-X receptor)
10	3c/d	TR/bacteria	16.3	29.3	27.5	52.4	activator (thyroid hormone receptor)
11	3e/f	Oct1/HeLa *	10.5	3.5	4.7	9	B cell specific activator
12	3g/h	Sp1/HeLa *	3.2	2.6	2.2	1.3	class II gene activator
13	4a/b	G4-94/bacteria	0.5	0.2	1	40.2	activator (DNA binding domain)
14	4c/d	G4-147/bacteria	3	0.1	0.1	8.7	activator (DNA binding domain)
15	4e/f	G4-AH/bacteria *	1.9	0.5	0.3	3.8	class II gene activator
16	4g/h	G4-VPI6/bacteria	1.5	1.3	0	0	class II gene activator
17	5a/b	G4-CTF/bacteria	0.9	0	0.1	8.2	class II gene activator
18	5c/d	G4-Sp1/bacteria	4.5	16.6	9.3	76.7	class II gene activator
19	5e/f	G4-E1A/bacteria	1.8	1.2	0	8	class II gene activator
20	5g/h	G4-IE/bacteria	4	0.8	0	0.9	class II gene activator
21	6a/b	G4-Tat/bacteria	2.3	1.6	3.4	15.7	class II gene activator
22	6c/d	PC4-P/HeLa *	5.4	100	77.5	14.5	coactivator (phosphorylated)
23	6e/f	PC4-N/bacteria	16.8	2.3	0.8	10.6	PC4 (C-terminal deletion)
24	6g/h	PC4-ΔS/bacteria	30.3	1.6	0.1	37.5	PC4 (N-terminal deletion)
25	7a/b	PC4-m1/bacteria	3.9	35	41.6	87	PC4 (CKII sites mutated)
26	7c/d	PC4-m2/bacteria	3.9	23.2	44.4	75.1	PC4 K231/K29A
27	7e/f	PC4-m2/bacteria	10.4	23.6	48.3	71.9	PC4 (K351/K41A)

28	7g/h	PC4-m3/bacteria	5.4	21.7	41.2	61.2	PC4 (R27A/K281/K29A)
29	8a/b	PC4-m4/bacteria	3.8	31.5	45	<b>100</b>	PC4 (R47N/K531/R59A)
30	8c/d	PC4-m5/bacteria	2.2	1.4	4.5	89.5	PC4 (F77P)
31	8e/f	PC4-m6/bacteria	2.8	37	66.7	87.3	PC4 (K29A)
32	8g/h	PC4-m7/bacteria	0.6	27.4	56.9	75.4	PC4 (K41A)
33	9a/b	PC4-wt/bacteria	2.8	37	66.7	72.7	transcriptional coactivator (wild type)
34	9c/d	p52/bacteria *	2.1	0.8	0	49.8	transcriptional coactivator
35	9e/f	p75/bacteria *	5	1.4	0	54.6	transcriptional coactivator
36	9g/h	p75-C/bacteria *	0	1.3	0.7	66.2	coactivator (C-terminal 326-530)
37	10a/b	p300-C/baculovirus *	4.6	11.6	11.3	14.5	transcriptional coactivator (1135-2414)
38	10c/d	PCAF/baculovirus *	2.5	2.4	14.5	<b>98.5</b>	histone acetyltransferase
39	10e/f	PCAF-C/baculovirus *	5.3	8.4	8	74.3	PCAF (352-832)
40	10g/h	TAF250/baculovirus †	1.7	1.1	0.6	10.9	transcriptional coactivator
41	11a/b	Topo I/baculovirus ‡	1.1	1	0.9	12.4	DNA unwinding/transcription
42	11c/d	Topo I/baculovirus ‡	4.7	2.4	2.1	12.6	Topo I (Y723F)
43	11e/f	Topo I/baculovirus ‡	5	1.6	1.1	6	Topo I (wild type)
44	11g/h	Topo I/HeLa	2.3	1.7	0.7	76.5	native Topo I *
45	12a/b	ASF/SF2/bacteria *	13.5	33.5	89	40.4	splicing factor (SR protein)
46	12c/d	SR/HeLa †	55	80	<b>100</b>	9.7	splicing factors (SR family)
47	12e/f	GST-Nu/bacteria **	<b>100</b>	0.4	3.3	8.3	pre-rRNA processing factor (nucleolin)
48	12g/h	GST-K/bacteria	2.8	0.4	1.5	1.8	negative control

\* Ge et al., *Methods Enzymol.*, 274, 57-71, 1996

† Chiang et al., *EMBO J.*, 12, 2749-2762, 1993

‡ Kershner et al., *J. Biol. Chem.*, 18, 34444-34453, 1998

§ Luo et al., *Cell*, 71, 231-241, 1992

¶ Jackson and Tjian, *Proc. Natl. Acad. Sci. USA*, 86, 1781-1785, 1989

|| Ge et al., *Proc. Natl. Acad. Sci. USA*, 91, 12691-12695, 1994

|| Ge et al., *Mol. Cell*, 2, 751-759, 1998

|| Ogryzko et al., *Cell*, 87, 953-959, 1996

|| Mizzen et al., *Cell*, 87, 1261-1270, 1996

|| Wang and Roeder, *Mol. Cell*, 1, 749-757, 1998

|| Pourquier et al., *J. Biol. Chem.*, 272, 26441-26447, 1997

|| Zahler et al., *Genes Dev.*, 6, 837-847, 1992

|| Valdez et al., *Mol. Immunol.*, 32, 1207-1213, 1995

\* Relative binding affinity of the specified probe to each target on the array, normalized to the highest signal for each probe.

\*\* TopoGEN Inc., Columbus, OH

The number, position (address), name/source (and related reference), affinities for each probe and known function for each of the 48 target polypeptides are indicated. The highest affinities of the individualized proteins for each probe molecule [GST-nucleolin for p52 (addresses 12e/f), PC4-P for the dsDNA (addresses 6c/d), SR for the ssDNA (addresses 12c/d) and PC4-m4 for the RNA (addresses 8a/b)] where normalized to 100 and are indicated in bold.

#### Example 7: Kits

UPAs as disclosed herein can be supplied in the form of a kit for use in molecule binding analyses. In such a kit, at least one polypeptide array is provided. The kit will also include instructions, usually written instructions, to assist the user in probing the array. Such instructions can optionally be provided on a computer readable medium.

Kits may additionally include one or more buffers for use during assay of the provided array. For instance, such buffers may include a low stringency, a high stringency wash, and/or a

stripping solution. These buffers may be provided in bulk, where each container of buffer is large enough to hold sufficient buffer for several probing or washing or stripping procedures. Alternatively, the buffers can be provided in pre-measured aliquots, which would be tailored to the size and style of array included in the kit.

5 Certain kits may also provide one or more containers in which to carry out array-probing reactions.

Kits may in addition include either labeled or unlabeled control probe molecules, to provide for internal tests of either the labeling procedure or probing of the UPA, or both. The control probe molecules may be provided suspended in an aqueous solution or as a freeze-dried or lyophilized powder, for instance. The container(s) in which the controls are supplied can be any conventional container that is capable of holding the supplied form, for instance, microfuge tubes, ampoules, or bottles. In some applications, control probes may be provided in pre-measured single use amounts in individual, typically disposable, tubes or equivalent containers.

10 The amount of each control probe supplied in the kit can be any particular amount, depending for instance on the market to which the product is directed. For instance, if the kit is adapted for research or clinical use, sufficient control probe(s) likely will be provided to perform several controlled analyses of the array. Likewise, where multiple control probes are provided in one kit, the specific probes provided will be tailored to the market. In certain embodiments, a plurality of different control probes will be provided in a single kit, each control probe being from a different class of molecules (e.g., a nucleic acid probe, a protein probe, a ligand probe, etc.).

20 In some embodiments of the current invention, kits may also include the reagents necessary to carry out one or more probe-labeling reactions. The specific reagents included will be chosen in order to satisfy the end user's needs, depending on the type of probe molecule (e.g., nucleic acid, polypeptide, or ligand) and the method of labeling (e.g., radiolabel incorporated during probe synthesis, attachable fluorescent tag, etc.).

25 Further kits are provided for the labeling of probe molecules for use in assaying arrays provided herein. Such kits may optionally include an array to be assayed by the so labeled probe molecules. Other components of the kit are largely as described above for kits for the assaying of UPAs.

30

In view of the many possible embodiments to which the principles of our invention may be applied, it should be recognized that the illustrated embodiments are only a certain examples of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

35



We claim:

1. A protein interaction assay comprising:  
contacting an array of substantially pure target polypeptide molecules stably associated with a surface of a solid support with a detectable probe molecule under conditions sufficient to produce binding; and  
detecting the binding.
2. The assay of claim 1, further comprising removing unbound probe molecule prior to detecting the binding.
3. The assay of claim 1, wherein the detectable probe molecule comprises a single-stranded nucleic acid, a double-stranded nucleic acid, a protein, or a ligand.
4. The assay of claim 1, wherein the detectable probe molecule comprises a tag useful for detection.
5. The assay of claim 4, wherein the tag is fluorescent, luminescent, or immunogenic.
6. The assay of claim 1, wherein the array comprises a microarray.
7. The assay of claim 1, wherein the polypeptides are associated with the support at discrete addresses.
8. The assay of claim 7, wherein each address contains only one substantially pure target polypeptide.
9. The assay of claim 1, wherein the binding detected is a binding pattern.
10. An assay to determine polypeptide-binding of a probe molecule, comprising:
  - (a) preparing a labeled sample of the probe molecule;
  - (b) contacting the labeled sample with an array of substantially pure target polypeptides stably associated with the surface of a solid support under conditions sufficient to produce binding;
  - (c) separating unbound labeled probe from the array to produce a probed array; and
  - (d) detecting the binding.
11. The assay of claim 10, further comprising contacting the probed array with at least one additional member of a signal producing system.
12. The assay of claim 11, wherein the contacting the probed array with at least one additional member of a signal producing system is prior to detecting the binding.
13. A universal protein array, comprising  
a plurality of substantially pure target polypeptide samples provided on a solid support,  
wherein the samples are immobilized on the solid support in an addressable pattern.
14. The array of claim 13, wherein each address contains only one substantially pure target polypeptide.

15. The array of claim 13, wherein the addresses are arranged in rows and columns.
16. The array of claim 13, wherein the array is arranged in a computer readable format.
17. The array of claim 13, comprising at least 10 different polypeptide samples.
18. The array of claim 13, comprising at least 30 different polypeptide samples.
19. The array of claim 13, comprising at least 100 different polypeptide samples.
20. The array of claim 13, wherein the array comprises a microarray.
21. The array of claim 13, wherein the solid support comprises glass, nitrocellulose, polyvinylidene fluoride, nylon, fiber, or combinations thereof.
22. The array of claim 13, wherein the polypeptides comprises transcriptional factors, transcriptional activators, or transcriptional coactivators.
23. The array of claim 22, wherein the polypeptides comprise TFIIA, TFIIB, TBP, f:TFIID, TFIIE, TFIIF, f:TFIIH, Pol II, RXR, TR, Oct 1, Sp1, G4-94, G4-147, G4-AH, G4-VP16, G4-CTF, G4-Sp1, G4-E1A, G4-IE, G4-Tat, PC4-P, PC4-N, PC4-C, PC4-ΔS, PC4-m1, PC4-m2, PC4-m3, PC4-m4, PC4-m5, PC4-m6, PC4-m7, PC4-wt, p52, p75, p75-C, p300-C, PCAF, PCAF-C, TAF250, Topo I (wt), Topo I (mt), Topo I (wt)\*, Topo I (nati), ASF, SR, GST-Nu, or GST-K.
24. A kit for determining polypeptide-binding of a probe molecule, comprising a polypeptide array; and instructions.
25. The kit of claim 24, wherein the instructions include directions for exposing the probe molecule to an array of substantially pure polypeptides on a support under conditions in which the probe molecule is capable of binding to one or more of the polypeptides of the support to detect biological interactions between the probe molecule and the one or more polypeptides.
26. The kit of claim 24, wherein the polypeptide array comprises a microarray.
27. The kit of claim 24, further comprising a buffer.
28. The kit of claim 24, wherein the polypeptide array comprises a plurality of substantially pure polypeptide samples.
29. The kit of claim 24, further comprising a probe molecule standard.
30. The kit of claim 29, wherein the probe molecule standard comprises a label.
31. The kit of claim 28, wherein the substantially pure polypeptides comprise transcriptional factors, transcriptional activators, or transcriptional coactivators.
32. The kit of claim 31, wherein the polypeptides comprise TFIIA, TFIIB, TBP, f:TFIID, TFIIE, TFIIF, f:TFIIH, Pol II, RXR, TR, Oct 1, Sp1, G4-94, G4-147, G4-AH, G4-VP16, G4-CTF, G4-Sp1, G4-E1A, G4-IE, G4-Tat, PC4-P, PC4-N, PC4-C, PC4-ΔS, PC4-m1, PC4-m2, PC4-m3, PC4-m4, PC4-m5, PC4-m6, PC4-m7, PC4-wt, p52, p75, p75-C, p300-C, PCAF, PCAF-C, TAF250, Topo I (wt), Topo I (mt), Topo I (wt)\*, Topo I (nati), ASF, SR, GST-Nu, or GST-K.
33. A method of analysis of protein-molecule interactions, comprising:  
obtaining a plurality of different substantially pure protein specimens;

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placing a sample of each specimen in a discrete addressable location on a recipient array; and

probing the array with a detectable probe molecule.

34. The method of claim 33, wherein the array comprises a microarray.

35. The method of claim 33, wherein the probe molecule comprises a nucleic acid, a polypeptide, a ligand, a fragment thereof, or mixtures thereof.

36. A method of analyzing a plurality of binding characteristics of an array of polypeptide samples, comprising:

(a) providing a protein array comprising a plurality of different polypeptide samples;

(b) exposing the protein array to a first probe that may interact with the samples of the universal protein array to identify those samples to which the first probe binds;

(c) detecting a first binding pattern of the first probe;

(d) repeating (b) through (c) with a second probe to identify samples to which the second probe binds.

37. The method of claim 36, further comprising stripping bound first probe from the array prior to exposing the array to the second probe.

38. The method of claim 36, wherein the protein array comprises a plurality of substantially pure target polypeptide samples; and a solid support,

wherein the samples are immobilized on the solid support in an addressable pattern.

39. The method of claim 36, wherein the first probe and the second probe are selected from different classes of molecules.

40. The method of claim 36, wherein the protein array comprises a microarray.

41. The assay of claims 1 or 10, wherein detection is automated.

42. The method of claim 36, wherein detection is automated.

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FIG. 1A

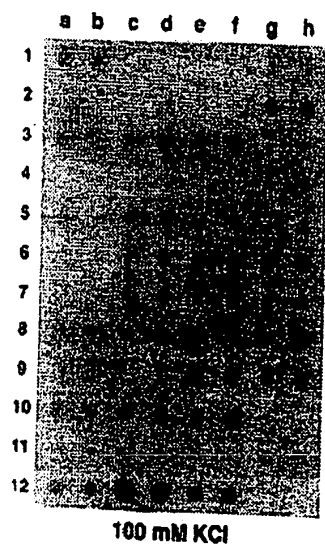
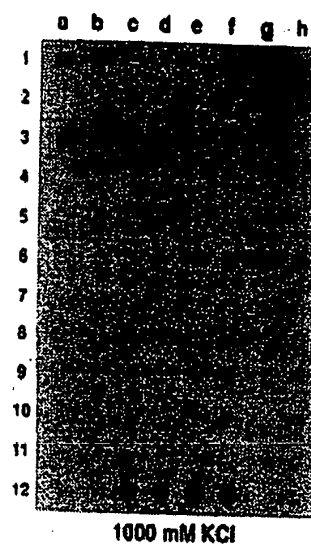
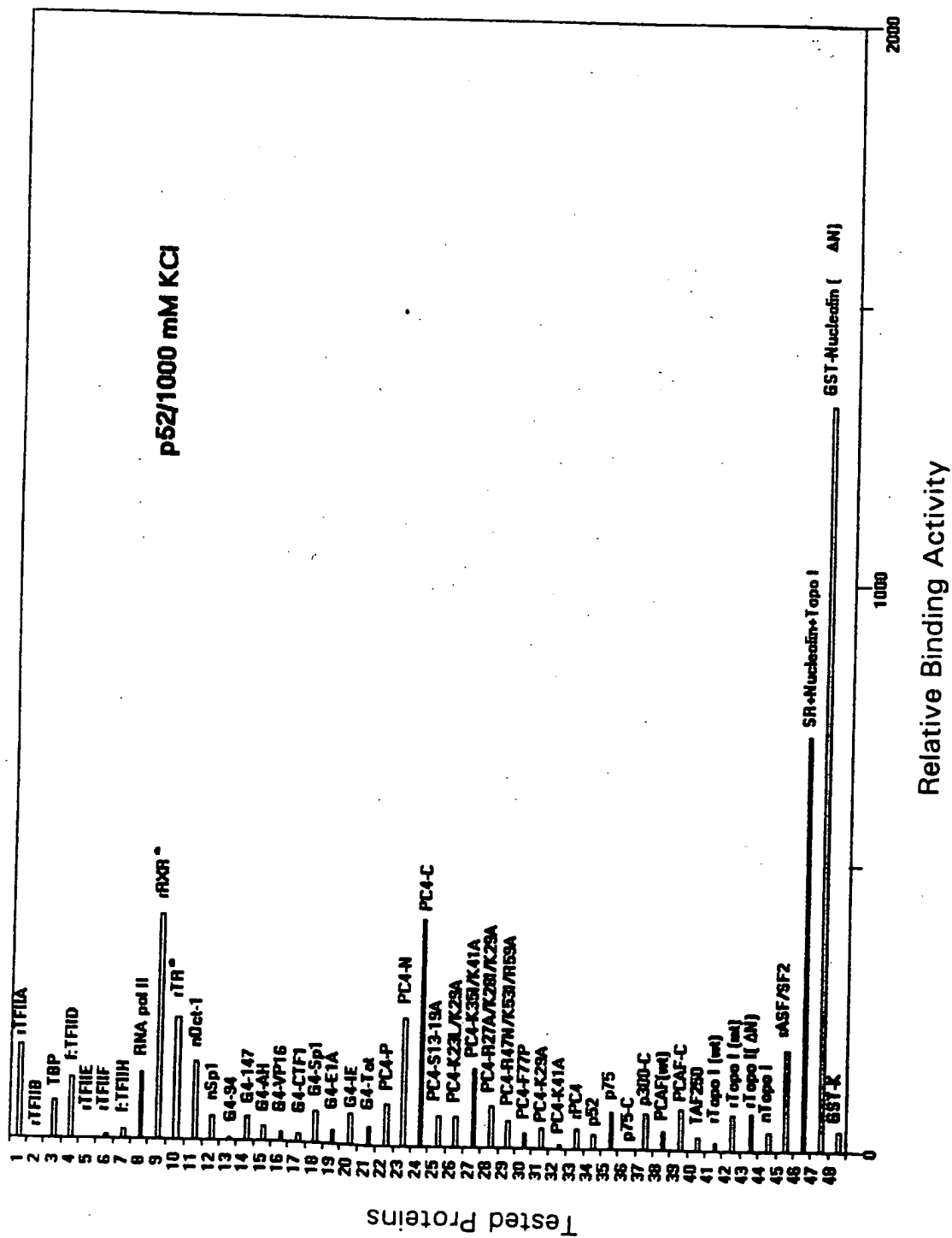


FIG. 1B



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FIG. 1C



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FIG. 2A

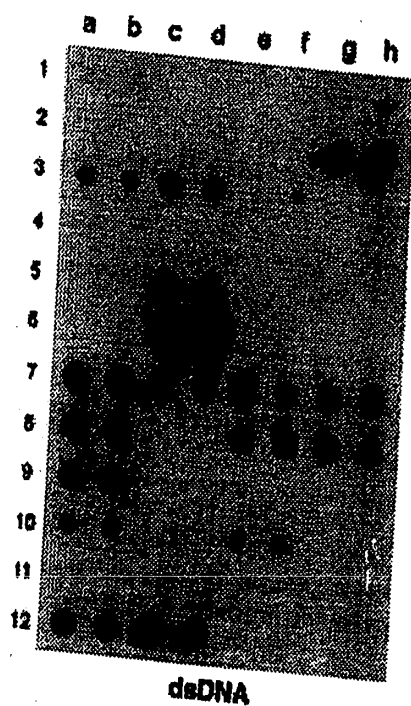


FIG. 2B

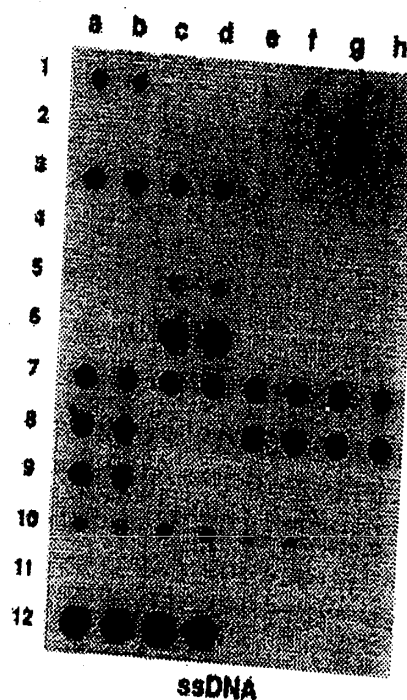


FIG. 2C

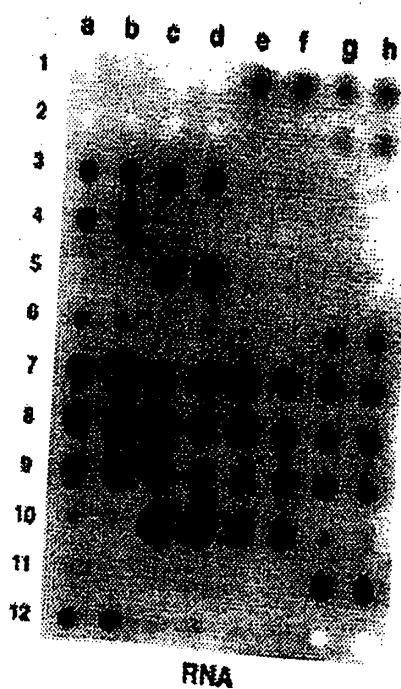
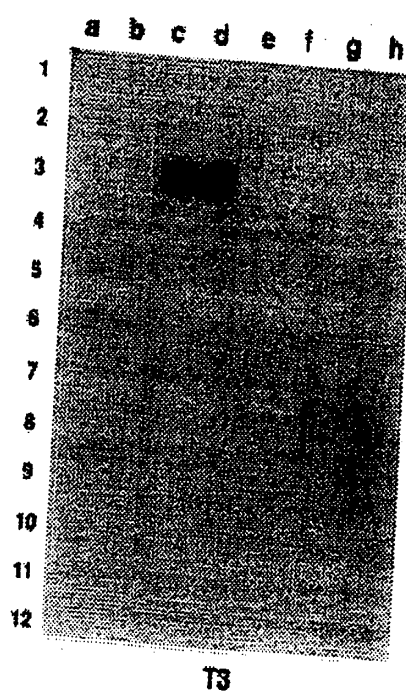


FIG. 2D



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FIG. 3A

	a	b	c	d
1	CTD	RPB5	RPB6	RPB8
2	RPB10 $\alpha$	RPB10 $\beta$	TBP	f:TFIID
3	RXR	TR	His-H1	Co-His
4	HMG1	ASF	GST-Nu	GST-K

FIG. 3B

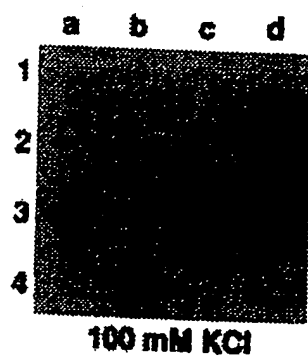
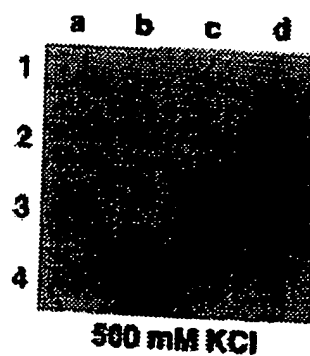


FIG. 3C







17. The array of Claim 1, wherein the organic thinfilm on the array comprises a monolayer.
18. The array of Claim 17, wherein the monolayer comprises a self-assembled monolayer comprising molecules of the formula  $(X)_aR(Y)_b$  wherein R is a spacer, X is a functional group that binds R to the surface, Y is a functional group for binding the protein-capture agent onto the monolayer, and a and b are, independently, integers.
19. The array of Claim 18, wherein both a and b are equal to 1.
20. The array of Claim 18, wherein:  
said substrate is selected from the group consisting of silicon, silicon dioxide, indium tin oxide, alumina, glass, and titania; and  
X, prior to incorporation into said monolayer, is selected from the group consisting of a monohalosilane, dihalosilane, trihalosilane, trichlorosilane, trialkoxysilane, dialkoxysilane, monoalkoxysilane, carboxylic acid, and phosphate.
21. The array of Claim 18, wherein the substrate comprises silicon and X is an olefin.
22. The array of Claim 1, wherein the substrate comprises a polymer.
23. The array of Claim 18, further comprising at least one coating between the substrate and the monolayer, wherein said coating is formed on the substrate or applied to the substrate.

24. The array of Claim 23, wherein:  
the coating is a noble metal film; and  
X, prior to incorporation into said monolayer, is a functional group selected from the group consisting of an asymmetrical or symmetrical disulfide, sulfide, diselenide, selenide, thiol, isonitrile, selenol, trivalent phosphorus compounds, isothiocyanate, isocyanate, xanthanate, thiocarbamate, phosphines, amines, thio acid and dithio acid.
25. The array of Claim 23, wherein the coating is titania or tantalum oxide and X is a phosphate group.
26. The array of Claim 1, wherein each protein-capture agent has been immobilized onto the organic thinfilm by an affinity tag.
27. An array of bound proteins, comprising:  
(a) the array of Claim 1; and  
(b) a plurality of different proteins which are expression products, or fragments thereof, of a cell or a population of cells in an organism, wherein each of said different proteins is bound to a protein-capture agent on a separate patch of the array.
28. A diagnostic device comprising the array of Claim 1.
29. A method of assaying in parallel for a plurality of different proteins in a sample which are expression products, or fragments thereof, of a cell or a population of cells in an organism, comprising:  
(a) delivering the sample to an array of spatially distinct patches of different protein-capture agents under conditions suitable for protein binding, wherein each of the proteins being assayed is a binding partner of the protein-capture agent of at least one patch on the array; and